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INTERFERTILITY BETWEEN TWO SYMPATRIC FISHES, *NOTROPIS LUTRENSIS* AND *NOTROPIS VENUSTUS*

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The redhorse shiner, *Notropis lutrensis* (Baird and Girard) and spottail shiner, *Notropis venustus* (Girard) are among the most abundant fishes in southwestern North America. They are considered to belong to separate subgenera, *Moniana* and *Erogala* respectively, by Hubbs (1955). The close relationship between the subgenera is shown by their adjacent position among the twelve subgenera of *Notropis* recognized by that author.

The two species do not occupy entirely identical geographic ranges. *Notropis venustus* is the more eastern in distribution. Populations are found from the Devil's River and the adjacent section of the Rio Grande, Texas, to the coastal streams emptying into the Gulf of Mexico near Houston. It is apparently absent from the Tamaulipan Biotic Province of Blair (1950). From Houston the range extends eastward in the coastal streams to the Apalachicola River in Georgia and Florida. Spottail shiners are found northward in the Mississippi River Valley to southern Illinois. The eastern and northern limits of the geographic range of the spottail shiner is taken from that given by Bailey, Winn, and Smith (1954). We have noted no pronounced morphologic variations of spottail shiners in those Texas populations we have studied. The northern limit of the range of *Notropis lutrensis* extends from southeastern Wyoming, through Nebraska, southeastern South Dakota, western and southern Iowa, to central Illinois. The eastern limit extends from central Illinois southward in small numbers along the Mississippi River to southeastern Mississippi, and thence in fresh and even slightly brackish water along the western Gulf of Mexico to northern Mexico. It has not been recorded from the Ozark Highlands. Small populations are found in sandy streams along the Arkansas River as it passes through the Ozarks. On the west the redhorse shiner ranges from southeastern Wyoming through central Colorado and central New Mexico to Mexico. The southern extent of the range, in northern Mexico, is incompletely worked out. The problem there is complicated by the presence of many isolated populations that may be conspecific with *N. lutrensis*. The variability shown by the isolated populations in Mexico is continued over the adjacent semi-isolated drainages in Texas and New Mexico. All of the individuals of the

three living samples from Trans-Pecos Texas that we have studied in the laboratory can be distinguished easily from all others. Toward the north and east, the redhorse shiners are less variable, with a coastal and a Great Plains morphologic form. Extremely dense populations occur throughout the Plains states of Nebraska, Kansas, and Oklahoma, as well as in the Plains regions of Texas. The sympatric range extends from southern Illinois southward to near the mouth of the Mississippi River, thence southwestward to the mouth of the Devil's River, and thence on a more or less straight line back to southern Illinois, but not including the Ozark Highlands. The total sympatric area approximates 120,000 square miles.

Natural hybrids between the spottail and redhorse shiners are found in many parts of their common geographic range. In addition to the general report of hybridization between the subgenera by Hubbs (1955), two local hybrid swarms have been reported. One of these hybrid swarms occurs in the Guadalupe River near Kerrville, Texas, with from 23% to 62% hybridity (Hubbs, Kuehne, and Ball, 1953). The high hybrid frequency occurs in that part of this clear spring-fed stream where wash-water from a gravel pit results in extremely turbid conditions. Another hybrid swarm has been noted in the San Marcos River southeast of San Marcos, Texas, by Jurgens (1951). During the 1920's the middle reaches of this stream were subjected to heavy pollution from the Luling oil field. It is likely that formation of the two hybrid swarms resulted from the two severe environmental disturbances. The San Marcos hybrid swarm may now be nearing extinction as no hybrids were obtained there in seven collections made during August, 1954, when a few individuals of each parental species were taken. However, a few hybrids were taken with both parental species in March, 1955. The Guadalupe hybrid swarm has been studied repeatedly during collections made from 1953 to 1955. There is an indication of a reduction in the number of hybrids following the construction of a reservoir at Kerrville. Other hybrid swarms have been noted in Illinois (Carl L. Hubbs, personal communication, 1955). As hybrid swarms have been noted in two distinct parts of the sympatric range, no localized racial tendency for loss of isolating mechanisms is suspected.

Both parental species have been maintained in the laboratory. Repeated spawnings have been observed. As the adults are voracious egg eaters and readily eat their own prolarvae (= sac fry) in clear aquaria, few young have been raised with their parents. We have not noted cannibalism of postlarvae (= advanced fry). Usually when numbers of young appear in stock tanks with their parents, the water contains much detritus kept in suspension by currents resulting from aeration. On two occasions we have placed males of one species with females of the other and recovered young after removing the parents. The first such experiment was set up on December 16, 1953, with males of *N. lutrensis* from the Sabine River near Tatum, Texas, and females of *N. venustus* from the Guadalupe River three miles west of (upstream from) the hybrid swarm. In addition five intraspecific controls were set up. On December 18 the breeding stocks were removed. On December 26 three young hybrids were observed. Two days later an additional hybrid was seen. Although the tanks were watched for ten additional days, no young appeared in any control tank. All four hybrids were raised to mature size. They were intermediate between the parental species, but varied among themselves. Three were males, two of which approached *N. lutrensis* and the other is more or less similar to *N. venustus*. The single female is intermediate. No young have appeared in the tank with the parents, although apparent breeding activity has been observed.

On January 28, 1954, males of *N. venustus* from Montell Creek in Uvalde

County, Texas, were placed in a tank with females of *N. lutrensis* from the Oklahoma side of Lake Texoma near the western limit of the range of *N. venustus* in the Red River system. Montell Creek is one of the Nueces River head-waters, which are inhabited by populations of a minnow, called *N. lepidus* (Girard) by Hubbs (1954), morphologically similar to and isolated from *N. lutrensis*. No controls were set up. Shortly after the parents were removed, young appeared in the tank. On May 6, 1954, they numbered 36. The hybrids appear to be intermediate between the parental types in shape and color. Only two of the 22 fish remaining on April 15, 1955, were males. During November and December of 1954 the two males and three of the females were isolated and fed heavily in a large aquarium. No young appeared although breeding activity was observed. On January 6, 1955, after the five fish had been returned to the stock tank, breeding activity was observed. The ripe female and both males were netted and the gametes were removed artificially and mixed. All three parents were returned to the stock tank undamaged. On January 10, 45 eggs were seen to be developing. Hatching occurred from January 13 to 15. The young developed as well as, or better than, our various inter- and intrapopulational crosses between members of the *N. lutrensis* complex carried out with the same techniques. On March 27, 1955, the vigorous young were changed from their hatching pan to an aquarium and during the next two or three days all but one F_2 died following symptoms similar to those of fish killed by cold shock. During the same weekend a "blue norther" suddenly ended unseasonable warm weather. As our laboratory room is not yet temperature-controlled, the room temperature dropped suddenly from 79 to 68° F. High mortalities of our experimental stocks and controls have occasionally occurred both when fish were moved to new tanks and following the sudden temperature drops during "northers." No other stocks died at this time, but none were subjected to identical conditions. The remaining F_2 hybrid has recovered from the ordeal and is as healthy as any of our controls. Another lot of eggs was stripped (before March 27) from the F_1 hybrids and development was noted. They were not maintained because of the lack of space and the fine condition of the then rapidly growing F_2's from the January 26 cross. Unfortunately no female in the F_1 hybrid tank has been noted as ripe since.

We have also made three back crosses with *Notropis lutrensis* and an additional one between the parental species. A female *N. lutrensis* from the Guadalupe River just downstream from the hybrid swarm area became ripe on April 10, 1955. Her eggs were mixed with sperm from one of the two F_1 hybrid males. Sixty-three fish of this backcross hatched. On April 17, 1955, the backcross was repeated with a female from the same stock. Ninety-three of them hatched. Both back cross stocks now consist of vigorous young. Two laboratory raised female *N. lutrensis* from the Lake Texoma stock became ripe on April 20, 1955. The eggs from one were mixed with sperm from one of the two F_1 hybrid males. Twenty of them hatched but all young were eaten by hybrids between the darters *Etheostoma spectabile* Agassiz and *Percina caprodes* (Rafinesque). The eggs from the second laboratory raised Lake Texoma female were mixed with sperm stripped from a *N. venustus* collected in Bernhardt Creek, Rusk County, Texas. Eighteen of these hybrids hatched and are vigorous. Since this paper was submitted, these fish have produced F_2 hybrids in the stock tank. No additional crosses of the above combinations have been attempted by stripping gametes.

Although we have not completed our studies on the relative fertility of various F_1 offspring from crosses between these two species and inter- and intrapopulational crosses within each, it appears that the interspecific hybrids are as successful

and as fertile as any others. Therefore, there appears to be no genetic barrier to interbreeding between these two species, at least between the members of the populations studied. Repeated simultaneous capture of young of both species indicates no seasonal reproductive isolation. Although the two species tend to occupy different macro- and micro-habitats, *N. venustus* in swifter water than *N. lutrensis*, there is approximately 25% overlap in wild-caught samples. The breeding sites selected in aquaria by different populations of the *N. lutrensis* complex vary from the gravel in the bottom of the tank to the vegetation floating on the surface, so selection of spawning site might be an isolating factor in certain regions. However, members of a single population from the Guadalupe River just east (downstream) from the hybrid swarm area have spawned both in the bottom corner of an aquarium and in floating vegetation. Various populations of *N. venustus* may likewise vary in selection of spawning site, but careful observations have not been made. An investigation of preferred breeding sites of the various species and populations and of their hybrids under differing aquarium conditions is planned. Thus either mate recognition or breeding site selection are suspected to be the major isolating factors in those regions where the two species do not interbreed. In the few areas where hybrid swarms occur, disturbances of the environment may well have broken these barriers between the species. Such disturbances often induce natural hybridization between species of plants and animals, including fishes (Hubbs, 1955). The turbid waters below the gravel pit at Kerrville and the pollution from the Luling oil field may upset species recognition during spawning in a manner similar to that which permits the survival of eggs and prolarvae in a turbid tank crowded with adults.

Acknowledgments

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ON THE EVOLUTION OF PARTHENOGENESIS IN LONCHOPTERA (DIPTERA)

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In recent years much time and thought has been devoted to answering the questions: what are the forces and mechanisms that promote evolutionary change, and how do they operate? The usual direct approach to this problem has involved study of forms which are believed to have undergone recent evolutionary change and may still be actively evolving. One indirect method of studying the problem would involve examination of those forms which are *not* believed to be evolving rapidly. By studying such stable species it may become possible to determine what series of events may block evolutionary change, and thus what must *not* happen if evolution is to occur. Probably the most extreme form of evolutionary stability in animals may be expected to occur among those species which are obligatorily parthenogenetic, and thus have lost the ability to produce genetically novel types through effective genetic recombination.

Such species are of special interest from two points of view. First, have they really entered an evolutionary blind alley as genetic orthodoxy would have us believe? Second, what conditions may exist which could lead to parthenogenesis in an originally bisexual form? This second problem is of general importance since parthenogenesis has arisen repeatedly in many different kinds of animals and plants. It is the purpose of this paper to report work carried out on the dipterous genera Lonchoptera and Drosophila which bears on these questions.

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PART I: OBSERVATIONS ON LONCHOPTERA DUBIA CURRAN

Lonchoptera dubia is a member of the family Lonchopteridae (Orthorrhapha, Brachycera). The family is monogeneric, and in the New World contains only five known species. Of these five, one is undescribed; the other four; *L. dubia, L. uniseta, L. occidentalis,* and *L. borealis,* were described by Curran (1934). Only *L. dubia* is considered to be parthenogenetic, since males are known in the other four species. The European members of the family have been reviewed by de Meijere (1906), who recognizes seven species, all of which are believed to be bisexual, with the possible exception of *L. furcata* in which males are almost never found. Of course the infrequent discovery of males in a given species is not of itself proof that it may not be partially or wholly parthenogenetic, since parthenogenetic flies may at times produce sterile males (Stalker, 1954b, 1956). In the case of *L. dubia,* in which males are unknown, Sturtevant (1923) has examined the seminal receptacles of wild-caught females and finds they are always empty, although eggs from such females may develop to hatching.

Lonchoptera dubia is northern in distribution, being taken as far north as southern Canada, and as far south as Los Angeles in the west, Colorado along the Continental Divide, Missouri in mid-continent, and the Great Smoky Mountains in the east. It is also reported from Argentina (Curran, 1934) and New Zealand (Harrison, 1950). Although adults of this species are almost always found associated with the *Trifolium repens–Plantago lanceolata* complex characteristic

of lawns, roadsides, and pastures, Dr. H. L. Carson has taken the species from wild grass in meadows in the Sierra Nevadas, and the author has taken it from similar habitats in the Rocky Mountains.

The adult food, as judged by crop examination, is chiefly or entirely nectar, and adults may be kept alive and in good condition in the laboratory for as long as seven months on a diet of honey and water. The larval food is unknown, but as judged by the long body hairs, the larvae are certainly not plant miners; they may possibly feed on the micro-organisms developing on damp dead leaves. In the laboratory, newly-hatched larvae have not taken any food except such micro-organisms, and even on this food have not completed development. Wild-caught females, taken when they are reproductively active, will in some instances oviposit abundantly in the laboratory, and will lay on almost any surface, wet or dry. Only a small percentage of wild-caught females will lay freely, however, and it has been found that 24-hour storage at 5–10° C. seems to act as a stimulus to oviposition. In our experience most of the the egg-laying in the laboratory occurs between the hours of 1 and 4 A.M.

Judging from external appearance and from the condition of the ovaries of wild-caught females, it appears that in the St. Louis area there are about three generations per year, and that further north (southern Manitoba) there are not more than two per year. Wild-caught females taken in the middle west later than September are usually in reproductive-arrest, but if held in the laboratory at 25° C. may begin to oviposit the following spring.

Wild flies were collected with a hand-net and ovary squashes made in aceto-orcein following treatment in 1 N HCl. It was found that females held in the laboratory (even under refrigeration) for more than three days made rather inferior chromosome preparations.

Description of the Chromosomes. The oogonial metaphase complement of *L. dubia* consists of four chromosomes: a pair of equal-armed and a pair of unequal-armed V-shaped chromosomes (see figure 1-J). Critical oogonial smears were obtained in 19 individuals coming from 14 widely separated localities, and all were diploid. In addition all evidence from the polytene chromosome smears indicated diploidy. Thus if polyploids exist in this species they are either rare or occur outside the range studied.

As indicated previously (Stalker, 1954a), the ovarian nurse-cell nuclei of *L. dubia* contain large banded polytene chromosomes similar to those found in the salivary glands of Drosophila. Pressure on these nuclei causes separation of the paired chromosomes into four different arms of unequal lengths, which may correspond to the euchromatic portions of the metaphase arms, although this is not known. There is no clear chromocenter as in some Drosophila species. Pairing in the mature nurse-cell chromosomes is characteristically loose or non-existent. Since no crosses can be made to standard stocks, as in Drosophila, comparison of the banding pattern of different individuals was made by comparing visually observed chromosomes with large series of photomicrographs of known types. This rather tedious technique is clearly unreliable to the extent that it may not reveal minor differences in banding pattern. Thus although at least thirteen different banding patterns were found in the four chromosome arms by this method, this number might conceivably be increased by a more exhaustive study. The conclusions to be drawn later in the paper would not, however, be affected by such an increase.

The four polytene chromosome arms were not all equally favorable material for study. The longest of these, arm IV, proved to be regularly heterozygous for an extremely complex series of rearrangements (see figure 1-f). Since this arm lacked good landmarks, tended to fragment under pressure, and showed close pairing only in immature, indistinctly

banded specimens, it was not studied in detail.

Of the other three arms, I, which was the shortest, was found to exist in four structurally different forms. These are indicated in figure 1-b, c, d, and are referred to as sequences A, B, G, and K. None of these sequences differed from the others by anything as simple as a single inversion. Although A and B do differ by an inversion in the middle third of the chromosome, other more complicated changes have taken place at the tips. The source of the differences between sequences B, G, and K could not be worked out by the photographic method, and they are clearly complex. Considering arm I alone, only three types of females were found to occur in nature, the heterozygotes A/B and B/K and the homozygote G/G.

In arm II four sequences were found: L, M, O, and M′. Of these four the only simple difference was that between M and M′, which appear to differ by a single inversion in the middle third of the chromosome. The differences between the other sequences are of the complex sort described above for arm I. Arm II is marked by a normally well-developed "puff," and with the exception of M and M′, the different sequences differ in the position of this puff. The four sequences were found in only four combinations: the homozygotes L/L and M/M and the heterozygotes M/O and M/M′. Sequence M is shown in figure 1-a and sequence L in figure 1-h. In these photographs the positions of the puffs are indicated by hollow arrows.

Arm III showed three sequences: P, P′, and Q illustrated in figure 1-e, h. P and Q differ by a sub-terminal inversion, and P′ differs from P by a short median inversion. These three chromosome types were found in only two combinations: P/Q and P′/Q.

As might be expected in a parthenogenetic species, the various chromosomal associations within arms were not randomly associated between different arms. In fact, on the basis of the three arms studied, the species could be subdivided into only four chromosomal races, as follows:

Race	Arm I	Arm II	Arm III
1	A/B	L/L	P/Q
2	B/K	M/O	P′/Q
3	B/K	M/M′	P′/Q
4	G/G	M/M	P′/Q

It will be noted that of the nine chromosome-arm combinations listed above only three, G/G, L/L, and M/M are homozygous. Thus it would appear that *L. dubia* is acting as a permanent heterozygote of some sort, with either lethality or non-production of the theoretically possible homozygotes. Since the great majority of established parthenogenetic animal species are apomicts, that is, have lost their normal meiosis, and essentially replaced it by mitosis, it seemed probable at first that *L. dubia* was an apomict, as this would readily account for the absence of homozygotes in populations where various types of heterozygotes abound. In order to check this point, the author and Dr. H. L. Carson began a study of Feulgen whole-mounts of freshly-laid eggs from wild-caught females. For this study only eggs from females held in the laboratory for 36 hours or less were used. Owing to the difficulty of getting enough eggs, the study was not completed, but it was made abundantly clear that the species is not apomictic; that meiosis is essentially normal, leading to the formation of four haploid nuclei; and that the early cleavage nuclei are again diploid. Reconstitution of diploidy by fusion of haploid meiotic products was not actually observed, but it seems virtually certain that it must occur, since any other method of restoring the diploid condition would lead to complete structural homozygosity.

Thus it may be concluded that *L. dubia* is an automict, with a fusion nucleus functioning as the cleavage nucleus. With this type of reproduction it would be expected that females heterozygous for structural rearrangements should produce some homozygous progeny, and that, therefore,

death might be an important factor in keeping the populations free of homozygotes. Actually there is a good deal of mortality in early development. A sample of 135 eggs from 31 wild Race-1 females (which had been in the laboratory at 25° C. for less than 12 hours, and hence were presumably in good condition) showed development to hatching in only 91 cases, partial development and death in 32 cases, and no development of any sort in the remaining 12. Thus about one-fourth of the progeny died, and yet since the mothers were heterozygous for both pairs of chromosomes, ¾ of their progeny might have been expected to show new homozygous combinations if there were random association of chromosomes through automixis. It is of course possible that ¾ of all progeny actually are inviable or sterile, and that a great deal of unobserved mortality exists in larval and pupal development. However, it seems inconceivable that such an obviously successful species could have evolved from and replaced its bisexual ancestor in the face of such high mortality. A more likely explanation is that the fusion of the haploid meiotic products is not a random one. In Lonchoptera, as in Drosophila, the four haploid nuclei are arranged in a straight line following the second meiotic division, and fusion of the two *central* nuclei (those coming from different secondary oocytes) would always reconstitute the original heterozygous karyotype of the mother if no proximal crossing-over occurred between the centromere and the heterozygous rearrangements. Thus the known 25% mortality in *L. dubia* eggs and embryos may represent homozygotes produced by fusions between nuclei derived from the same secondary oocyte, or produced by crossing-over and fusions between *central* nuclei. It should be emphasized that both of the above types of nuclear fusions are known in other parthenogenetic species. In *Drosophila parthenogenetica* both central and terminal nuclear fusions occur with equal frequencies (Stalker, 1954b) but in the Coccid, *Lecanium hesperidum* the nuclear fusion occurs regularly between the terminal nuclei (Suomalainen, 1950).

Geographical Distribution of the Chromosomal Races. Table 1 and figure 2 clearly indicate differences in distribution for the four races. In the Missouri, Illinois, Iowa, and Minnesota areas only Race 1 has been found. In Manitoba, Ontario, Quebec, and Colorado both Race 1 and Race 2 are found. In the Appalachian uplift, Races 3 and 4 occur, while in Rochester, New York, all four races are found. Samples from Tuolumne Co., California, collected by Dr. H. L. Carson, included Races 2, 3, and 4.

These sharp differences in distribution are an indication of physiological and genetic differences between the races. Further evidence for such genetic differences is based on the following observation: not only may different races occur in the same general area, but in a great many instances they actually coexist in a space no larger than five or six square feet, and may be taken together in a single swing of the net. Table 1 lists 10 collecting sites furnishing two or more races: not itemized in this table are 11 additional collecting sites in the Great Smoky Mountains with two races each. Thus individuals of different races are, so to speak, rubbing shoulders in nature. These races are, of course, completely discrete: due to lack of males, there can be no question of balanced polymorphism of the type found in single populations of bisexual Lepidoptera and Drosophila. This means that unless the different races differ in their ecological requirements, that is finally in their genotype, they must compete. But continued competition in so many localities is highly improbable to say the least; one race should replace all others in a given region. The fact that this does not occur indicates that the races do not compete, and are physiologically, ecologically, and thus genetically different.

Altitudinal Distributions of the Chromosomal Races. With such good evidence

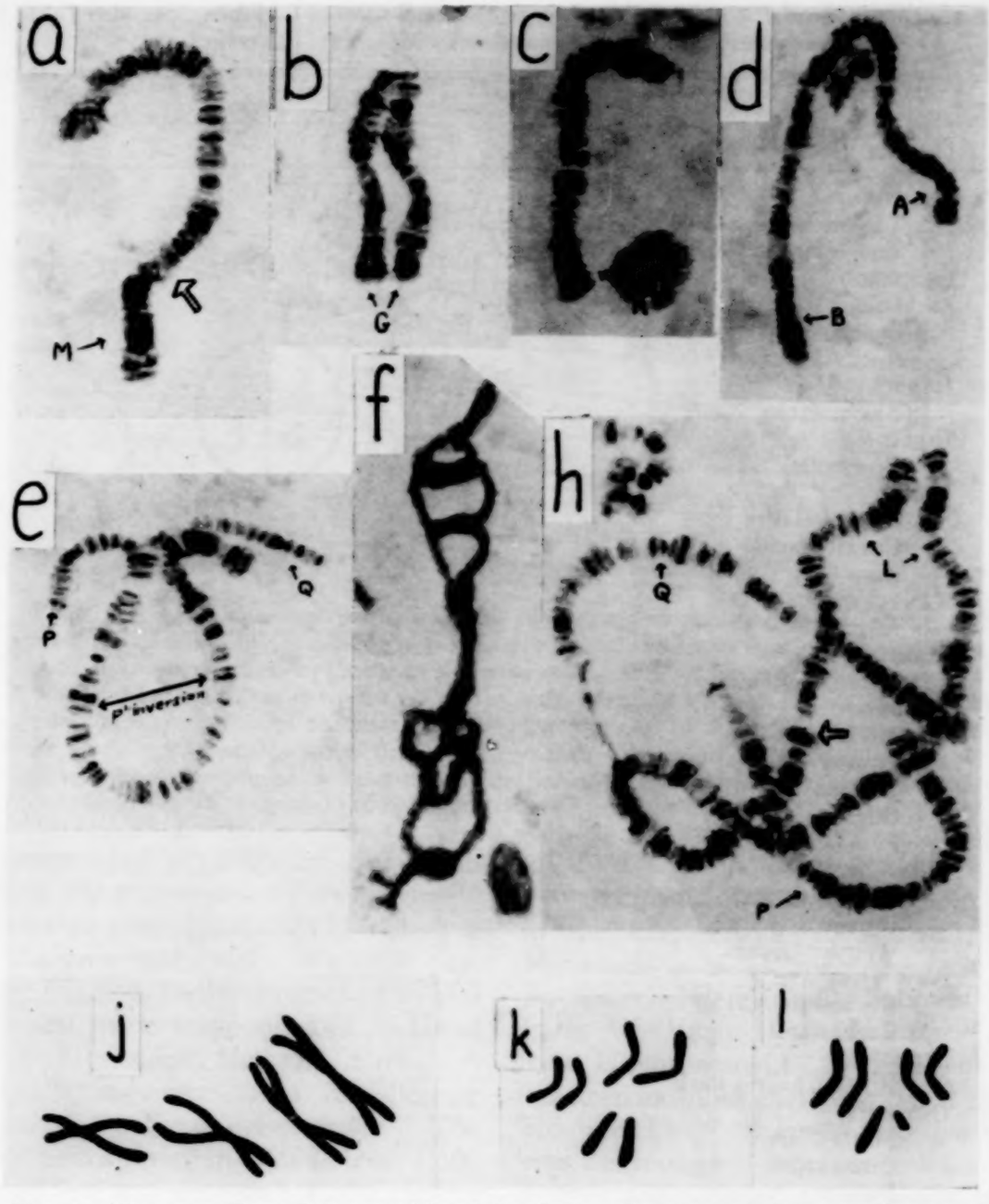

FIG. 1. Figures a through h are of banded polytene chromosomes from ovarian nurse cells of *L. dubia*. Figures j through l are of metaphase chromosomes from *L. dubia* and *L. occidentalis*.

a. Single homolog, sequence M of Arm II, Race 4; Greenville, Ind.
b. Homozygous sequence G/G of Arm I, Race 4; Gatlinburg, Tenn.
c. Single homolog, sequence K of Arm I, Race 3; Rochester, N. Y.
d. Heterozygous sequences A/B of Arm I, Race 1; Evansville, Wis.
e. Heterozygous sequences P/Q of Arm III, Race 1; Evansville, Wis. In this figure the limits of the inversion by which sequence P′ differs from both sequences P and Q are shown.
f. Heterozygous sequences of Arm IV, Race 2; Madison, Wis., taken from immature nurse-cell nucleus.
h. Homozygous sequence L/L of Arm II and heterozygous sequences P/Q of Arm III, Race 1; Davis Corners, Ia.
j. Oogonial metaphase of *L. dubia,* Race 1; Davis Corners, Ia.
k. Oogonial metaphase of *L. occidentalis;* Rocky Mt. Nat. Pk., Colo.
l. Spermatogonial metaphase of *L. occidentalis;* Tuolumne Co., Cal.

TABLE 1. *Geographical distribution of the four chromosomal races of* L. dubia. See also Table 2 for further details concerning Great Smoky Mts. Nat. Pk. collections.

Locality	Races 1	2	3	4
CALIFORNIA				
Tuolumne Co. (4,800′)		1	1	17
Tuolumne Co. (8,200′)				3
COLORADO				
Drake	1	1		
Loveland	3			
Rocky Mt. Pk. (9,000′)	1			
ILLINOIS				
Bloomington	1			
INDIANA				
Greenville				1
IOWA				
Davis Corners	4			
Crawfordsville	5			
Iowa City	1			
KENTUCKY				
Kilgore (632′)				2
MANITOBA				
Winnipeg Beach	6	12		
MICHIGAN				
Mackinac City	7			
MINNESOTA				
Jackson	1			
Olivia	10			
Pine Island	4			
MISSOURI				
St. Louis	5			
University City	3			
Webster Groves	9			
NEW YORK				
Rochester-1 (515′)	2		1	
Rochester-2	2	1		5
OHIO				
North Chagrin Park				2
ONTARIO				
Marten River	1			
Ottowa		1		
Bissett	3	1		
QUEBEC				
Broughton Junction	8			
Rigaud	1			
Bouchette	3	2		
Quebec City	1			
TENNESSEE AND NORTH CAROLINA				
Great Smoky Mountains Park			39	61
VIRGINIA				
Lexington (945′)				8
Amherst				1
Keysville				1
WEST VIRGINIA				
Babcock State Park (1,100′)			1	9
WISCONSIN				
Madison-1	2			
Madison-2	2	1		
Evansville	6			
St. Croix Falls	4	4		
Totals	96	24	42	110 = 272

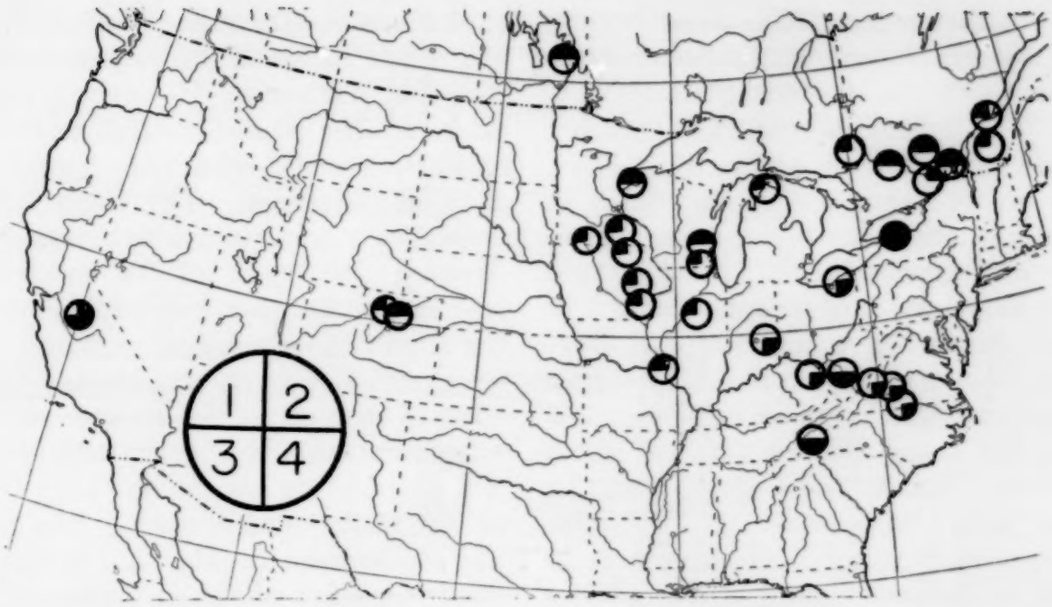

FIG. 2. Geographical distributions of the four chromosomal races of *L. dubia*. The symbol for each locality is divided into four quadrants, with each quadrant representing a given chromosomal race, as shown in the enlarged symbol on the map. Blackening of a given quadrant indicates the presence of the corresponding chromosomal race. For example: The symbol for California indicates the presence of Races 2, 3 and 4 but not Race 1, while that for Mackinac City, Mich., indicates the presence of Race 1 only. The base map here reproduced is from the Goode Series of Base Maps, number 102 (1937). It is published with permission of the University of Chicago Press.

for genetic and physiological differences between the chromosomal races, it might be expected that altitudinal differences in distribution would exist. With this possibility in mind, in the summer of 1954 a study was made of populations in Great Smoky Mountains National Park. A total of 93 specimens from 21 collecting sites were analyzed cytologically. The different sites varied in altitude from 1,500 to 6,300 feet, and were on both the eastern and western slopes of the main north-east south-west ridge. All specimens proved to be Race 3 or Race 4. The data are summarized in Table 2 and indicate that there is no simple relationship between race-frequency and altitude. The general picture which emerges may be summarized as indicating that both races are found in all areas and at all altitudes sampled, with site-to-site differences in frequency no greater than expected by chance. This seeming independence of altitude and race-frequency is somewhat surprising when one considers the differences in geographical distribution of the two races involved. As indicated in table 1, only two specimens of Race 3 have been taken in the east outside the Great Smoky Mountains area (1 specimen each from Rochester, N. Y., and Babcock State Park, W. Va.). Race 4, on the other hand, is represented by 29 specimens from 8 eastern localities excluding Great Smoky Montains Park. It seems clear that whatever the ecological requirements for Race 3, they are satisfied at all levels in the

TABLE 2. *Summary of altitudinal distributions of Races 3 and 4 in the Great Smoky Mountains National Park.* From 1 to 9 flies were taken from each collecting site; of the 18 sites yielding 2 or more flies, 11 showed both Race 3 and Race 4.

Altitude	Number of		Per cent Race 3	Per cent Race 4
	Sites	Flies		
1,500′–2,200′	6	20	40	60
2,750′–3,100′	4	15	47	53
4,000′–4,600′	5	26	69	31
4,900′–6,300′	6	32	9	91
Totals	21	93	39	61

park, but they are rarely satisfied outside of it. No really peripheral populations of *L. dubia* exist in the Smoky Mountains area. The Triofolium-Plantago complex is found at all altitudes and so is *L. dubia*. It is possible that in higher mountains, in which real altitudinal limits exist, differences in frequencies of the two races might likewise be observed. Such frequency changes have been found in *Drosophila robusta* genotypes and karyotoypes in these mountains, but in the latter species a real upper limit occurred at about 4,000 feet (Stalker and Carson, 1948).

The clear dependence of Trifolium-Plantago on clearings is of importance in regard to the past history of *L. dubia* in these mountains. This fly is almost certainly lacking in the forests; prior to the cutting of roads it must have been completely absent from the higher mountains unless present as isolated populations in the high-altitude grass balds. In pioneer days these balds were extensively used for cattle grazing, and during this period may well have supported Trifolium-Plantago and Lonchoptera. A visit was made to one of the larger grass balds (Andrew's) from which Trifolium-Plantago has been reported in recent years, and both *Trifolium repens* and *L. dubia* were found at the edge of the bald along a foot trail, but not out on the bald proper. In pre-pioneer days it is apparently uncertain whether the balds were used by Indians for summer camp grounds. Unless they were so used, it seems probable that Lonchoptera is a recent immigrant in the higher Smoky Mountains.

Morphological Differences in the Chromosomal Races. The genus Lonchoptera is taxonomically difficult because of the lack of morphological differentiation between species. Thus it is not surprising to find great morphological similarity between the four chromosomal races. The most obviously variable intraspecific character is the ground-color of the thorax and abdomen. However, even if this character is genetically determined, it is clearly not associated with any particular chromosomal races, since both the black and yellow forms and all intermediates occur in all four races. However, a meristic study of 224 pinned specimens from 22 localities revealed what does appear to be a racial difference. The character concerned is a wing-vein index obtained by dividing the length of the sub-terminal segment of the fifth longitudinal vein by the length of its terminal segment. Such wing-vein indices were obtained for one wing of each of the pinned specimens, the wing-vein segments being measured under a dissecting microscope with the aid of an ocular micrometer. The results are summarized in table 3. In this table the frequencies of the chromosomal races are given on the right side; these frequencies are based on the data given in extenso in table 1. It will be noted that in the eastern part of the range the high indices are generally associated with populations that include Races 3 or 4, while the low indices are associated with Race 1-2 populations. Since the Rochester, N. Y., samples included all four races they will be disregarded for the moment. An analysis of variance based on a comparison of the remaining 16 eastern localities in the Race 1-2 area with the 4 localities in the Race 3-4 area indicates that there is no significant heterogeneity within either area, while the difference between the means of the two areas (1.37 versus 1.55) is highly significant (F-ratio = 55.19; F-ratio at 1% level of significance = 8.28). Thus, on the basis of the eastern data, it appears that Race 1-2 populations have a significantly lower wing-vein index than Race 3-4 populations.

The possibility that this difference in wing-vein index is environmental rather than genetic cannot of course be entirely excluded, although this seems highly improbable when one examines the pattern of the geographic variability in the index. It will be noted that while there is essentially no change from Missouri north through Iowa, Wisconsin, and Minnesota to Manitoba, and as far east as Quebec, an abrupt change takes place south of

TABLE 3. *Summary of wing-vein indices for* L. dubia *from various geographical localities.* The frequencies of the four chromosomal races are taken from the more extensive data given in table 1.

Locality	Number of flies	Mean wing-vein index	Frequencies of chrom. races			
			1	2	3	4
Great Smoky Mts.	25	1.59 ± 0.04			39	61
Lexington, Va.	11	1.57 ± 0.06				8
Babcock State Pk., W. Va.	15	1.51 ± 0.04			1	9
Kilgore, Ky.	4	1.41 ± 0.01				2
Rochester, N. Y.	7	1.51 ± 0.05	4	1	1	5
Quebec and Broughton Jct., Que.	8	1.37 ± 0.06	9			
Bouchette, Que.	6	1.37 ± 0.04	3	2		
Marten River, Bissett and Pembroke, Ont.	7	1.26 ± 0.04	4	1		
Winnipeg Beach, Man.	11	1.37 ± 0.04	6	12		
St. Croix Falls, Wis.	5	1.39 ± 0.04	4	4		
Evansville, Wis.	10	1.44 ± 0.04	6			
Madison, Wis.	12	1.38 ± 0.03	4	1		
Pine Island, Minn.	9	1.43 ± 0.04	4			
Olivia, Minn.	25	1.33 ± 0.02	10			
New London, Minn.	4	1.40 ± 0.02				
Windom and Jackson, Minn.	7	1.37 ± 0.03	1			
Crawfordsville and Iowa City, Ia.	21	1.37 ± 0.02	5			
Davis Corners, Ia.	9	1.35 ± 0.02	4			
Walker, Ia.	8	1.42 ± 0.03				
Emmetsburg, Ia.	4	1.39 ± 0.03				
St. Louis, Mo., and Bloomington, Ill.	5	1.27 ± 0.04	18			
Loveland and Drake, Colo.	11	1.50 ± 0.18	4	1		

Rochester, N. Y. The fact that the high indices are associated with chromosomal races found primarily in the Appalachian Mountains might suggest that the high wing-vein index is somehow a direct environmental effect associated with the high-altitude, especially so since the Colorado sample, taken from altitudes of 5,000 to 7,000 feet, shows a high index despite the fact that it includes Races 1 and 2. However, this explanation meets the difficulty that the low-altitude populations from Kentucky, Virginia, West Virginia, and New York also have a high wing-vein index. Moreover, within the Great Smoky Mountains National Park there is no apparent correlation between altitude and wing-vein index. Thus for the eastern populations, it appears probable that the observed association between race and wing-vein index has some sort of direct genetic basis.

The exclusion of the small Colorado sample, which does not fit the above interpretation, is based on its variability, as indicated by its unusually high standard error and its geographical position. Whether *L. dubia* exists in the great plains between Missouri and the Rocky Mountains is not known, but the possibility exists that the western forms of the species are genetically quite different from those in the east. In any case, the Colorado sample makes it doubtful that the racial differences in wing-vein indices apply to the western part of the species range.

The Origin of Parthenogenesis in L. dubia. Parthenogenesis in *L. dubia* is probably of recent origin as indicated both by the fact that all of the other species in the genus (with the possible exception of the European *L. furcata*) are apparently bisexual, and by the fact that in *L. dubia* itself parthenogenesis is imperfect, with

only about 90% of the eggs starting development.

Any hypothesis designed to explain the origin of parthenogenesis in this species must take into account the occurrence of the four chromosomal races. Their existence means that either we must assume extensive chromosomal and genic change subsequent to the loss of bisexuality or must assume that when males were lost all or most of the present-day chromosomal and genetic variability was somehow incorporated into the derived unisexual strains. It is clear that we cannot exclude entirely the possibility that extensive evolutionary change, and race formation, has occurred subsequent to loss of bisexuality. However, the theoretical difficulties inherent in this explanation are such that the alternate explanation seems much more probable. Thus let us consider how parthenogenesis might have evolved in such a way as to initially incorporate a great deal of chromosomal variability into the unisexual derivatives.

Two principal modes of origin are possible. Either a single all-important mutant gene arose which made it possible for carriers to reproduce efficiently without males, or a polygenic system was gradually built up by selection which made facultative parthenogenesis possible. These alternatives are obviously not mutually exclusive.

The evidence in other animals for a mutation conferring parthenogenetic ability in a single step is not at all clear. Hertwig's (1920) case of a strain of *Rhabditis pellio* which suddenly displayed parthenogenesis, presumably based on mutation or recombination, is probably not relevant, since in that species, as in related nematodes, pseudogamy existed, with the continuing need for stimulation of the egg by sperm, even though the sperm took no further part in development. If we grant the possibility of such a single all-important gene mutation as the source of parthenogenetic ability in *L. dubia,* it would still have to be assumed that the mutant gene concerned was widely disseminated in the bisexual populations prior to loss of males, in order to account for the extensive present-day chromosomal variability.

Evidence for the existence and gradual development of a polygenic system of modifiers conferring parthenogenetic ability is much clearer. In many orthopterous and lepidopterous species, and in at least four species of Drosophila, bisexual races show infrequent but successful development of unfertilized eggs to form fertile diploid females. That this facultative parthenogenesis is susceptible to improvement under selection is apparently demonstrated in *Solenobia triquetrella* (Seiler, 1942) and is clearly shown in *Drosophila polymorpha* and *D. parthenogenetica* (Stalker, 1954b). In the last species continued selection for parthenogenesis resulted in a gradual increase in the rate of parthenogenesis over many generations, and hence was probably based on a polygenic system.

The possible advantages of established parthenogenesis over bisexual reproduction are clear enough. Development of parthenogenesis may result in a two-fold increase in reproductive potential since only half as many parents are needed per individual. Even *L. dubia,* if only two-thirds of its eggs developed into normal adults, could reproductively out-strip a similar bisexual form. Such an increase in reproductive potential might be expected to be especially valuable to a species such as *L. dubia,* which is typically northern in its distribution, since survival of a colony through the winter would probably be related to the total size of the colony following the relatively brief summer breeding period. An additional advantage of parthenogenesis would be the fixation of the genotype, which would eliminate the experimental production of the largely ill-adapted new genotypes, characteristic of bisexual reproduction. Finally parthenogenesis in *L. dubia* is clearly associated with extensive genetic heterozygosity, and probable heterosis.

Regardless of the *ultimate* beneficial

effects of established parthenogenesis, what continued selective forces will ensure its development in the face of competition with the bisexual forms? In the case of *L. dubia,* what is known of the ecology of the species seems to offer a clue. As noted above, this species has been taken in the east only in association with the Trifolium-Plantago complex. Yet the present abundance of this complex in the New World is largely the result of man's activity. Prior to the opening up of North America by the white man with his parks, cemeteries, pastures, and roadsides, the Trifolium-Plantago complex must have existed in small semi-isolated habitats. Thus it is reasonable to suppose that in the past *L. dubia* populations were likewise small and semi-isolated. Such small populations would be subject to sampling error in regard to the sex-ratio, with repeated shortages of males. Under the influence of such male shortage, a given population would be strongly selected for any genotypes promoting development of unfertilized eggs. Gradual selective development of such genotypes would in turn increase the intensity of selection, since the more frequent the development of unfertilized eggs, the smaller the percentage of males produced, and the greater the opportunity for male-deficiency or complete absence by sampling error.

It is worthwhile pointing out in this connection that the major portion of such selection for parthenogenesis must almost certainly occur among bisexual populations. This is because once a population becomes permanently unisexual, even if temporarily protected from competition with bisexual populations by isolation, its rate of selective improvement will rapidly approach zero, since the only genetic variability available for selection is the heterozygosity contained in single females (or fortuitous new mutations). Thus an isolated population with a low level of parthenogenesis which by chance became unisexual would probably be unable to achieve any major increase in rate of parthenogenesis by selection. However if isolation between such small populations were not complete, then the advantageous effects of selection on periodicially male-deficient populations could be combined with the beneficial renewal of genetic variability by crossing between populations. This mechanism of the origin of parthenogenesis should lead to the simultaneous and gradual development of parthenogenesis in many sub-populations over a wide area. This would in turn readily allow for the incorporation of great genetic and chromosomal variability into the derived unisexual strains. Eventually as the high frequencies of parthenogenesis resulted in fewer and fewer males being produced, chance loss of males should occur in one population after another until all were gone. At this point further extensive improvement in rate of parthenogenesis should be difficult or impossible, due to the drying-up of genetic variability, and the species might be left with a yet imperfect parthenogenetic mechanism such that some of the unfertilized eggs would fail to undergo development. *L. dubia* is in fact in just this position, at least in so far as Race 1 is concerned, since as indicated above, approximately 9% of the eggs of wild-caught females failed to show any sort of development. Thus in this form, despite its obvious success as an unisexual species, the fertility is reduced not only by the apparent production of inviable homozygotes, but by occasional complete breakdown of the parthenogenetic mechanism in some eggs.

Part II. Chromosomal Variability in Bisexual Species of Lonchoptera

If, as suggested above, the chromosomal races of *L. dubia* owe their structural differences to variability left over from their bisexual ancestors, then it might be expected that present-day bisexual species of Lonchoptera might show some of the same sort of chromosomal rearrangements as those now found in *L. dubia.*

The only bisexual species readily obtained in quantity is *L. occidentalis.* That

this species is bisexual is indicated by the following facts: males and females are collected in equal numbers, dissections of males show normal testes and apparently mature sperm, copulations occur readily in the laboratory, and in the male the sex-chromosomes are markedly heteromorphic. As indicated by figure 1-k, l, the metaphase chromosomes of the oogonial and spermatogonial nuclei are markedly different from those in *L. dubia*. *L. occidentalis* is known from Alaska, Idaho, California, and Colorado. Dr. H. L. Carson kindly collected specimens from Tuolumne Co., California, some of which were squashed when collected and some sent to St. Louis for preparation. The author obtained a number of additional specimens from Rocky Mountain National Park in Colorado.

An examination of the ovary smears of seven specimens from the California collections indicated that of these seven, five were structurally homozygous throughout, while each of the remaining two specimens showed two tandem inversions in one chromosome arm. In the eight specimens studied from Colorado, four showed single inversions in one chromosome arm, two showed two inversions in one arm and one inversion in another, while the remaining two specimens showed single inversions in one arm and a complex consisting of at least four inversions (two overlapping) in another arm, this latter suggestive of the extremely complex arm IV of *L. dubia*. All preparations of this species showed much more consistent and intimate pairing of homologs than that shown in *L. dubia*. It should be emphasized that such examination of wild females, without photographic standards of comparison, could indicate only variability *within* individuals, and would not give an adequate indication of the chromosomal variability within or between populations.

Thus, in the only bisexual relative of *L. dubia* about which we have any information, complex structural heterozygosity of the type postulated for the bisexual ancestors of *L. dubia* does in fact exist.

Part III. Selection Experiments in Drosophila

For some years the author has been conducting an experiment to test the efficiency of selection for parthenogenesis in a unisexual strain of *Drosophila parthenogenetica*. In this species the unisexual strain was established in 1951, and has been maintained since that time, now being in its 80th generation. This species, being automictic, had available for selection only the genetic heterozygosity within single females, and apparently the available heterozygosity had gradually and completely disappeared by the 17th generation. Thereafter, despite continued selective pressure no further improvement had occurred by generations 53 and 62. If the cessation of selective improvement is indeed due to homozygosity, then outcrossing with bisexual strains, and reselection by establishment of unisexuality might be expected to bring about further improvement. Unfortunately only one original wild strain of the species was available, and it was from this strain that the unisexual line was derived. Thus any crossing between the closely-related bisexual and unisexual strains might be expected to bring about only minor selective advances thru recombination. Although the outcrossing experiments are not completed, and will be fully reported later, the results so far seem so pertinent to the present discussion that they will be briefly described.

Females from the unisexual strain (generation 55) were crossed to males from the bisexual strain and the F_1 allowed to breed bisexually and produce an F_2. From the F_2 bisexual generation virgin females were obtained to set up a new unisexual strain which then underwent three generations of unisexual selection for parthenogenesis. This procedure was carried out in 32 replicates. After two such consecutive backcrosses and re-extractions of unisexual lines, three of these which appeared to be most fertile were selected for five unisexual generations and

one of the three showed a marked increase in the rate of parthenogenesis as compared to the 53rd and 62nd generations of the unisexual strain, as indicated in table 4.

In table 4 the percentages in the body of the table are for the most part based on pooled contributions of all females, such pooled data being treated as single samples. In the case of the data for Unisexual Generation 53, and that for the unisexual strain extracted after backcrossing to the bisexual strain, the percentages given are the unweighted means of the performances of the individual females; the standard errors of these means are based on the sum of squares among females. The mean performance of each female was estimated from a sample of 250 to 700 unfertilized eggs. An analysis of variance shows that the unisexual strain derived following backcrossing differs significantly from the unisexual strain Generation 53, both with respect to the percentage of eggs starting development and to the percentage completing it. Thus approximately eight generations of unisexual selection alternated with unselected bisexual reproduction have accomplished what approximately 45 generations of continuously selected unisexual reproduction have failed to do.

These experimental results in Drosophila, in so far as they may be applied in a general way to other automictic parthenogenetic forms, clearly show that selective improvement of a character ultimately stops following loss of bisexuality, even though this character is clearly susceptible to rapid selective change in early unisexual generations and after the restoration of bisexuality. In this sense it indeed appears that the completely parthenogenetic strain has entered an evolutionary blind alley.

To sum up, the most reasonable hypothesis to account for the origin of parthenogenesis in *L. dubia,* keeping in mind its imperfect nature and the existence of the chromosomal races, is that it occurred by a gradual selection for a polygenic complex favoring development of unfertilized eggs, and that this gradual selection was promoted by temporary male shortages in small, semi-isolated populations, these small populations extending over a considerable portion of the species' range.

Summary

1. A study of chromosomal variability in the diploid parthenogenetic species *Lonchoptera dubia* Curran (Lonchopteridae, Brachycera, Diptera) is described. This study is based on an analysis of 272 females from 32 widely separated areas, chiefly in eastern North America.

2. *L. dubia* is almost entirely nearctic

TABLE 4. *Frequency of development of unfertilized eggs in* Drosophila parthenogenetica. The percentages and standard errors in lines 5 and 7 of the table represent the unweighted means of the performances of individual females. The mean performance of each female was estimated from a sample of 250 to 700 unfertilized eggs. All other percentages in the table are based on the pooled contributions of all females, such pooled contributions being treated as single samples.

Source of virgin females	Number of females observed	Number of eggs observed	Per cent eggs starting development	Per cent eggs completing development
Bisexual strain	218	74,538	0.91	0.08
Unisexual strain				
Generation 2	82	20,751	1.48	0.31
Generation 9	69	17,740	2.22	0.37
Generation 17	42	12,464	8.20	1.55
Generation 53	42	16,950	7.97 ± 0.66	1.56 ± 0.15
Generation 62	47	11,282	8.21	1.56
Second extraction after backcross to bisexual strain	12	4,604	20.52 ± 1.08	3.06 ± 0.26

and northern in distribution, occurring in the northern United States and southern Canada. It is collected by sweeping, principally from the White Clover-Plantain complex characteristic of pastures, lawns, etc.

3. In this species the nuclei of the mature ovarian nurse cells contain large, banded polytene chromosomes similar to those of Drosophila salivary glands. There are four polytene chromosome arms, and the banding patterns of three of these were studied in detail. On the basis of the eleven known sequences for these three arms the species may be divided into four chromosomal races which show distinctly different, but overlapping, geographical ranges. In many localities two or more chromosomal races were taken with a single sweep of the collecting net.

4. The four chromosomal races are permanent structural heterozygotes for some or all chromosome arms. Study of Feulgen whole-mounts of freshly-laid eggs indicated that *L. dubia* is automictic (meiotic), with two meiotic divisions followed by a fusion of two of the four haploid meiotic products. The absence of adult structural homozygotes in wild populations is probably explained by death during early development (occurring in at least 25% of eggs) and by possible fusion of second-division meiotic products derived from different secondary oocytes, which if occasional crossovers are disregarded, would exactly reconstitute the original heterozygosity of the mother.

5. In the eastern part of the range small but significant differences in the wing-vein dimensions occur between the populations made up of Races 1 and 2 and those made up of Races 3 and 4.

6. The micro-distributions of the various chromosomal races make it clear that they are not wholly competitive. If they were, one race would replace others in a given micro-habitat. Thus the four races are chromosomally, ecologically, and genetically different.

7. Selection studies on the automictic (normally bisexual) tychoparthenogenetic species *Drosophila parthenogenetica* indicate that continued selection for parthenogenesis within a unisexual line for 62 generations results in selective improvement in parthenogenesis during the first 17 generations, and thereafter no change in rate of parthenogenesis. However, crossing between the unisexual line and an ancestral bisexual one brings about marked selective advance in rate of parthenogenesis.

8. It is suggested that in *L. dubia* parthenogenesis has arisen gradually within extensive semi-isolated small populations of the bisexual ancestor. In such small populations sampling error would bring about recurrent male-shortages, resulting in selective advantage to parthenogenetic development. In time, as genotypes permitting parthenogenesis were built up in the small populations, the male-shortages would become increasingly acute and selection would be intensified. Crossing between different populations would allow more effective synthesis of parthenogenetic genotypes, at the same time "freezing" into the developing unisexual races much of the chromosomal variability originally present in the bisexual ancestor. Finally when all males were lost, further selective improvement in parthenogenesis might be slowed down or stopped, leaving the species with an imperfect parthenogenetic mechanism. Additional support for this hypothesis comes from the fact that the bisexual species, *L. occidentalis*, does have complex structural variability of the sort postulated for the bisexual ancestor of *L. dubia*, and also from the fact that parthenogenesis in *L. dubia* is imperfect, with about 9% of the eggs failing to show any development.

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RE-EXAMINATION OF THE DIVERSITY IN *PARTULA TAENIATA* [1]

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The studies made by Professor Crampton (1932) on the Partulae of Moorea have long furnished material for speculation about the evolutionary processes occurring in these snails. Of particular interest has been the diversity of traits which sometimes occur in the snails occupying adjacent valleys or regions. No plausible explanation could be offered for this diversity other than that of the operation of random genetic drift. The present study is an attempt to re-examine this diversity using recently developed biometrical methods. Such a study was made possible by the wealth of data published by Crampton.

Crampton directed most of his attention to the wide-spread species, *P. taeniata*, the one to be examined here. He described the past evolution of this species as a process whereby new varieties originate by isolation in southern Moorea, cross the previously isolating barriers and dislodge the progenitor varieties in the north. The migration of these varieties across the mountain ridges of Moorea has been clearly indicated by the geographical distribution and the physical relationships of the varieties.

He also observed that the snails inhabited the heavily vegetated valleys and were relatively isolated by the intervening mountain ridges. The genetic divergence shown by some of these intra-valley groups has suggested to some students of evolution that random genetic drift had been affective within these groups, at least it has been difficult to account for the divergence in any other way (see for example Huxley, 1942 and Dobzhansky, 1951).

In the present study, we shall concentrate our attention on the geographical subdivisions of the primary varieties and the primary varieties themselves. The subdivisions, which will be termed "subvarieties" in this paper, have been fully described by Crampton. The subvarieties are not equivalent to the intra-valley groups, for the territories of the former encompass several valleys each. However, it is felt that much of the observed genetic divergence may be accounted for at this slightly higher level. At the same time, a clearer understanding of the evolutionary processes which have been operating within this species may be obtained.

In order to deduce what forces might be effecting the observed divergence of geographically located groups, we may look for trends appearing in the phylogenetic relationships of the groups. The search for such trends in the present case consists of making comparisons of the phylogenetic relationships with the geographical relationships. Such comparisons should lead to a decision as to the major cause, or causes, of the observed genetic divergence. For example: if the phylogenetic relationships of the groups are shown to be randomly distributed on the island, genetic drift would be indicated—at least no evidence would be provided to disallow it; if the phylogenetic relationships were greater for geographically proximal groups, then a system of isolated groups showing occasional migration between adjacent ones would be indicated; if the phylogenetic relationships were less for geographically proximal groups, then the

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development of reproductive isolating mechanisms would be indicated.

One possible measure of phylogenetic relationship between groups would be an index which incorporates the differences between the group means of many variables. However, the redundancy introduced by incorporating correlated variables would invalidate the resulting index. A method of ridding the variables of such redundancy prior to combining their differences in an index has been developed by Mahalanobis (see Rao, 1952) as the "generalized distance" concept. The indexes, or "distances," so computed represent the degree to which the mean phenotypes of any two groups differ; the greater the distance, the less the groups are alike. Since these distances may represent phylogenetic relationships of the groups, they are referred to in the present paper as "phylogenetic distances."

A more appropriate measure of phylogenetic distance would be based upon genetic values rather than upon phenotypic ones, as has been done here. However, phenotypic values are the only ones available for this study and since the environment has relatively little effect (according to Crampton), it is felt that their use will not invalidate the observations.

Materials and Methods

The species, *P. taeniata,* has been divided into six "primary varieties" (terminology of Crampton). Further subdivision of these varieties was made by Crampton on the basis of their geographical location. These geographical subdivisions are the basic groups used below in the phylogenetic comparisons and will be referred to in this paper as "subvarieties." The names of the varieties and the abbreviations of the subvarieties as used here are shown in table 1. Their geographical locations are shown in figure 1, a modification of a map published by Crampton (1932, plate 15).

Fortunately for this investigation, Crampton has published data on the shell

Table 1. *Abbreviations of varieties and subvarieties of* P. taeniata

Abbreviation	Variety and geographical subdivision
P	*propinqua*
Sp-w	*spadicea*—western
Sp-e	*spadicea*—eastern
N	*nucleola*
St	*striolata*
E-o	*elongata*—outer series
E-i	*elongata*—inner series
E-r	*elongata*—Rotui sector
Si-ow	*simulans*—outer western series
Si-iw	*simulans*—inner western series
Si-oe	*simulans*—outer eastern series
Si-in	*simulans*—inner northern series
Si-on	*simulans*—outer northern series
N P	No Partulae

dimensions found in these varieties and subvarieties. These dimensions are, namely, shell length, shell width, aperature length and aperature width, which appear in the tables of "color classes" in the supplemental part of Crampton's paper. These data are sufficient for the calculation of the phylogenetic distances between the varieties and/or subvarieties. The *intra-varietal* correlation coefficients and standard deviations calculated from these data are shown in table 2. Appropriate weighting was made for the numbers of individual measurements included in each mean value given in the table on "color classes." Orthogonalizing and normalizing the varietal and subvarietal means (i.e., changing the variables into an uncorrelated set with zero mean and with standard deviation units) were accomplished by the use of these statistical estimates according to the procedure outlined by Rao (lit. cit.). (The varietal and subvarietal means were derived solely from the 1919, 1923 and 1924 data, whereas the correlation coefficients and standard deviations were derived from the data of all years given.) Phylogenetic

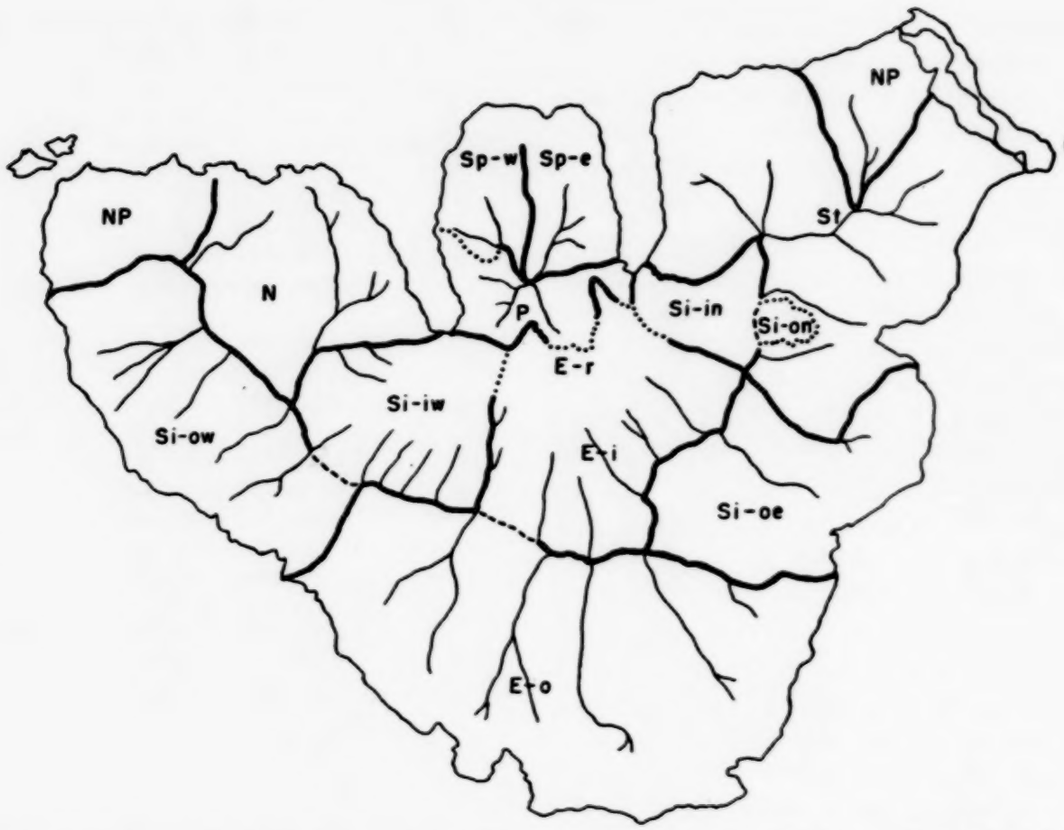

FIG. 1. Distribution of the varieties and subvarieties of *P. taeniata* on the island of Moorea. See table 1 for legend of abbreviations. Light lines are mountain ridges, heavy lines are boundary lines of territories of the varieties or subvarieties, dashed lines are mountain passes which have allowed migration at various times, and dotted lines are regions of overlap or uncertain boundary. (Modified from Crampton. Used by permission of the Carnegie Institute of Washington.)

distances were then computed using these orthogonal, normal mean measurements of the varieties and subvarieties according to the following formula:

$$D = \sqrt{(\bar{x}_{1a} - \bar{x}_{1b})^2 + (\bar{x}_{2a} - \bar{x}_{2b})^2 + \cdots (\bar{x}_{na} - \bar{x}_{nb})^2}$$

where $\bar{x}$ is a group mean of one of the orthogonalized variables, 1,2, . . . n, and D is the distance between the groups, a and b. The distances calculated here are in a four dimensional hyperspace, since there were four variables concerned.

The inter-varietal and subvarietal phylogenetic distances are given in table 3.

TABLE 2. *Intra-varietal correlation coefficients, standard deviations and grand means*

Measurements (mm)	Shell length	Shell width	Aperature length	Aperature width
Correlation coefficients:*				
Shell width	0.647			
Aper. length	0.923	0.643		
Aper. width	0.555	0.559	0.609	
Standard deviations:**	0.3353	0.3225	0.4830	0.3016
Mean measurement	15.773	9.162	8.513	6.329

* Degrees of freedom . . . 401.
** Degrees of freedom . . . 402.

TABLE 3. *Phylogenetic distances between varieties or sub-varieties of* Partula taeniata. (See table 1 for legend of abbreviations.)

P												
Sp-w	4.64											
Sp-e	2.68	2.09										
N	4.00	1.88	2.03									
St	4.61	2.20	2.35	1.58								
E-o	4.90	3.48	3.87	3.24	4.26							
E-i	6.14	4.79	5.40	4.47	5.78	2.10						
E-r	6.20	4.02	3.85	3.91	5.00	1.53	1.33					
Si-ow	3.54	1.33	1.02	1.79	2.01	3.32	4.97	4.32				
Si-iw	3.32	4.07	3.27	2.61	3.70	2.84	3.29	3.86	3.40			
Si-oe	3.40	1.94	1.78	2.26	3.10	2.31	3.89	3.42	1.35	2.97		
Si-in	4.01	3.23	3.30	2.91	4.30	1.69	2.27	2.40	3.08	2.55	1.92	
Si-on	5.95	3.89	4.78	3.50	4.79	2.02	1.31	1.12	4.27	3.51	3.46	2.15
	P	Sp-w	Sp-e	N	St	E-o	E-i	E-r	Si-ow	Si-iw	Si-oe	Si-in

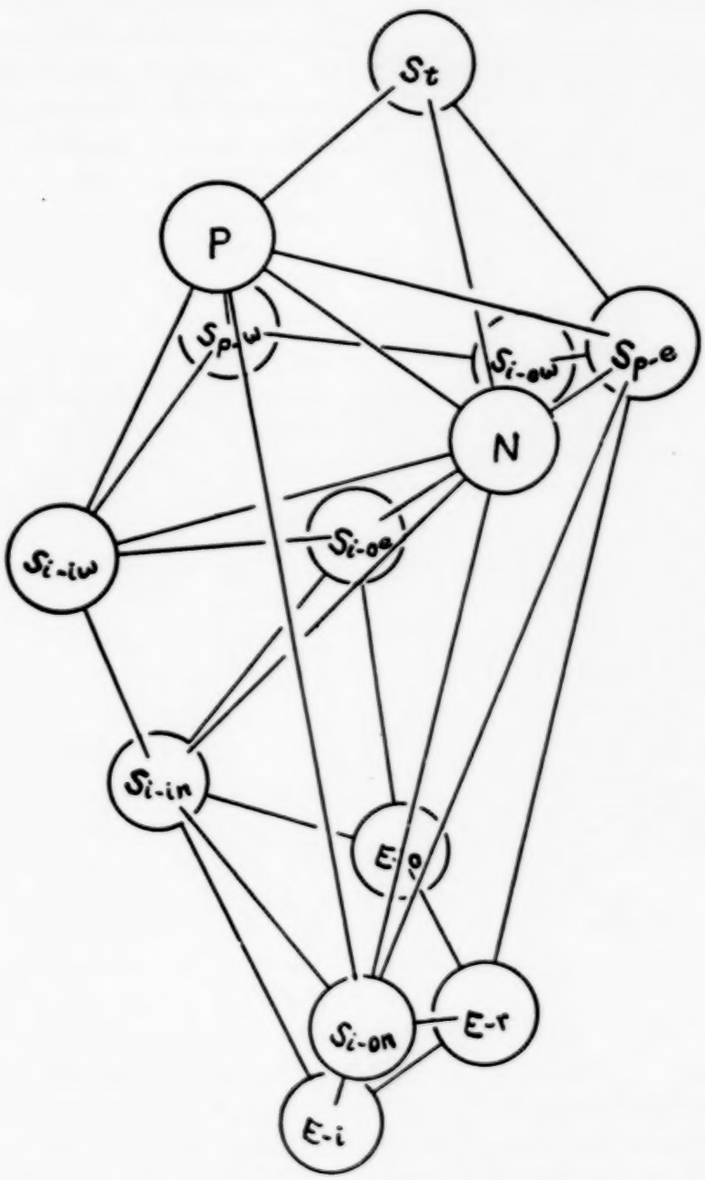

FIG. 2. A three-dimensional representation of the phylogenetic distances between the varieties and sub-varieties of *Partula taeniata*. See table 1 for legend of abbreviations.

The comparison of the values in this table with the map locations in figure 1 provides the information with which the conclusions have been reached. However, in order to aid in the visualization of the phylogenetic distances, the distances derived in the four-dimensional space have been reduced to a three-dimensional representation of the distances in figure 2. Not too much information is lost by doing so, however distances in table 3 should be the final criterion in making decisions about phylogenetic distances.

Observations and Conclusions

Crampton concluded that there were three basic groups of *P. taeniata* which differed in their times of origin. The oldest group was that of the (hypothetical) ancestral variety of the *nucleola, spadicea* and *striolata* varieties which he referred to as *primordia.* It is suggested that *simulans* originated from an isolated population of *primordia* in the outer southern valleys of Moorea, then expanded, replacing *primordia* in all but the three northern points of land. This process isolated the three derived varieties of *nucleola, spadicea* and *striolata,* leaving *simulans* to occupy the expansive southern regions. *Elongata,* he concluded, originated in a manner similar to that of *simulans;* however this time *simulans* was the progenitor variety and also the variety displaced in the subsequent northward migration. Apparently southern valleys of the outer region were sufficiently isolated—at least during some periods—to allow new varieties to become established. *Propinqua* originated somewhat later and in a different manner as an offshoot of *spadicea.*

These general relationships of the varieties postulated by Crampton are supported by the relationships seen in figure 2. The three northern varieties, *nucleola, spadicea* and *striolata,* are not far apart; *simulans* is intermediate in position and most variable; while *elongata* is at the opposite end of the *simulans* range from that of the northern varieties; *propinqua* is not far from *spadicea.*

When we look beyond these general interrelationships of the varieties, and examine the interrelationships of the subvarieties, we notice a persistent pattern, one that pervades the whole set of phylogenetic distances. The subvarieties of a variety (which has been so subdivided) may be classified as invaders or non-invaders when based on Crampton's postulations of invasions. We then see that the invading subvariety is always phylogenetically more distant to the displaced or preinhabiting variety (or subvariety) than is its related, non-invading subvariety. For example, the inner *simulans* subvarieties (Si-iw and Si-in) show a decided increase in phylogenetic distance to the *nucleola, spadicea* and *striolata* varieties when compared to the other *simulans* subvarieties (Si-ow and Si-oe). The inner *elongata* subvarieties (E-i and E-r) show increased phylogenetic distances to the northern varieties as well as from *simulans* when compared to the outer *elongata* subvariety (E-o). *Propinqua* also has become decidedly more distant to the inner *elongata* and *simulans* subvarieties (E-i, E-r, Si-iw and Si-in) than does its progenitor, *spadicea.* In every case that may be postulated, the invading subvariety becomes more divergent. These observations may be interpreted as a tendency for genetic divergence to occur whenever a variety invades a preinhabited territory, a divergence which distinguishes the invading subvariety from the preinhabiting variety.

If we agree with the foregoing observation then we might also suspect that the corollary would be true, i.e., that the non-invading subvarieties, those which remain in isolated territories, may indicate their varieties' progenitors by phylogenetic proximity. Apparently this is so. The subvarieties of *simulans* which have remained isolated from the northern varieties have not moved, phylogenetically, far from the postulated progenitor, *primordia* (represented by the northern varieties)

(i.e., Si-ow and Si-oe remain close to the northern varieties in figure 2). Similarly the more isolated *elongata* subvarieties are not distant from their postulated progenitor, simulans (i.e., E-o remains close to *simulans* subvarieties). Thus, the conclusions reached by Crampton about the origin of the varieties are corroborated by this new criterion. In addition, this corollary concurs with Crampton's observation that the northern varieties, the earliest varieties of *P. taeniata* on Moorea, resemble *P. filosa* of Tahiti, the most probable progenitor of *P. taeniata*.

Without first-hand familiarity with the material, we must avoid concluding that the entities which Crampton has described as primary varieties are truly species. However, the observations in this study indicate that the varieties are extremely close to being reproductively isolated. The few instances where the geographical distributions of the varieties overlap, the phylogenetic distances are relatively great (e.g., P and E-r, Si-on and St, Sp-w and P; according to Crampton; overlap in their distributions). Thus, the varieties or incipient species are on the verge in several places of becoming distinct enough to occupy identical territories. Perhaps, these varieties are going through the same processes of evolution that other species of *Partula* underwent in more remote times.

Discussion

The genetic divergence of these snail varieties and subvarieties may most easily be interpreted as a strong tendency for reproductive isolating mechanisms to be developed in a variety (or subvariety) upon entering a new territory. Such a tendency would concur with existing theories of the development of reproductive isolation (see Dobzhansky, 1951). In this particular case, the invading variety would be visualized as hybridizing with the preinhabiting variety to a limited extent. This would enable natural selection to build up mechanisms to limit further production of these (assumed) less adaptive hybrids.

It is difficult to conjecture just what traits may enter into promoting reproductive isolation in snails. Perhaps shell shape (essentially defined by the variables used here) may affect the reproductive mechanisms *per se,* or promote variety-recognition. However, if this does not seem plausible, some pleiotropic or linkage relationships with reproduction may be assumed.

The above observations suggest a way, other than random drift, by which the divergence found between the valley groups might arise. A more dynamic process than that of random drift may be envisaged as occurring among these groups of snails, a process which consists of the varying grades of interactions between adjacent groups. We may visualize some groups hybridizing, others developing reproductive isolating mechanisms and still others vacillating between the two extremes. In this way divergence without apparent pattern might be developed. Admittedly this is pure speculation based on the above observations on subvarieties; however it is offered here as an alternative to the often assumed and also speculative explanation of random drift.

Upon due consideration, the contention that random drift is operating in these groups of snails is difficult to defend, for the conditions have not been shown to be sufficient for random drift to be effective. First, the population sizes are relatively large for the operation of chance fixation of genes. The samples which Crampton obtained from these valleys averaged several hundred specimens per valley. The actual population sizes must be several times this number, for surely he did not exhaust the valley populations by his sampling. Second, the traits examined by Crampton have not been shown to be neutral or lacking in selection pressure (see Sheppard, 1954). Surely if a quantitative trait such as the shell shape examined in this paper is indicated to have

some adaptive value, then traits such as direction of coiling, banding patterns and coloration may just as plausibly have adaptive values. Third, the degree of isolation between the adjacent valleys is not always very great as suggested by the past migrations of these snails.

Regardless of whether random drift occurs to any appreciable extent or not, the observations are sufficiently persistent to warrant the conclusion that divergence occurs in varieties undergoing or which have undergone invasion and that this is most likely due to the development of reproductive isolating mechanisms. These observations are sufficient to further conclude that this process is a relatively important factor in the evolution of these snails and that it may explain the divergence found in various traits of the many varieties and species residing in what otherwise appears to be a uniform environment.

Summary

Shell dimensions of *Partula taeniata*, compiled by Professor Crampton, were used to calculate the "phylogenetic distances" between varieties and/or geographic subvarieties. The evolutionary processes previously postulated by Crampton were corroborated by the analysis of these distances. The relationships between phylogenetic distances and geographical locations were examined to determine a possible explanation of the observed genetic divergences of the varieties and subvarieties. It was repeatedly observed that the portion of a variety invading and displacing a preinhabiting variety becomes phylogenetically more distant from the displaced variety than does the non-invading portion of the invading variety. This observation was interpreted as the development of reproductive isolating mechanisms between the invading variety and the preinhabiting variety. Random genetic drift is therefore not accepted as the general explanation for the divergent traits of these snails occupying the same or adjacent territories. The observations on the divergence in traits considered here can be fully explained by natural selection for reproductive isolating mechanisms.

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GENE FREQUENCY IN SMALL POPULATIONS OF MUTANT DROSOPHILA [1]

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INTRODUCTION

Studies of gene frequency changes in laboratory populations have for the most part been concerned with the systematic effects of selection and with discovery of the nature of the selective differences involved between genetic alternatives. The classical experiments of L'Heritier and Teissier (1934, 1937) with common mutants, the later work of Dobzhansky (Wright and Dobzhansky 1946), and that of Wallace (1948) on naturally occurring genetic variants are examples of such studies applied to rather large populations maintained in population cages of the sort developed by L'Heritier and Teissier (1933).

Such experiments as these were carried out under conditions in which changes in gene frequency due to sampling accidents (random drift) were negligible. In the work of Reed and Reed (1948, 1950), Ludwin (1951) and Merrell (1953) on selective differences between mutants and wild type, non-systematic effects of considerable magnitude and attributable to accidents of sampling occurred; however, these data did not permit a close evaluation of the extent of this influence.

An experimental design in which both random and systematic pressures are brought into play in an orderly fashion and the values of the several theoretical parameters can be ascertained was originated by Kerr (Kerr and Wright, 1954). The method consists in observations on the changes in gene frequency between generations in each of a large number of small cultures in which the size of the breeding population is controlled. Each initial culture founds a line in which each successive generation is inititated with a random sample of uniform size taken from among the flies of the preceding generation. The form is that of a fairly large experimental population divided into a number of very small completely isolated subunits.

The present study was intended to provide information on the relative selective values of two mutant autosomal alleles and their several zygotic types under several different experimental conditions and to observe the effect of the interaction of selection and random drift on the distribution of gene frequencies among the elements of subdivided populations of the sort observed by Kerr.

MATERIALS AND METHODS

Stocks

The stocks used were obtained from strains of mutants at the brown locus of *Drosophila melanogaster* developed by Slatis (1955). The bw^{75} strain was derived by him in June of 1950 by x-irradiation of males from a scarlet stock which was very closely related to an "isogenic" stock of *bw*; *st*. This *bw*; *st* stock had been made "isogenic" by a marker-chromosome technique followed by over 40 generations of brother-sister mating. The scarlet stock at the time of irradiation was derived from a series of cultures stemming from an outcross of *bw*; *st* to *st* at the time homozygosity was initially enforced in the former. This outcross was followed by repeated backcrosses of single heterozygous females (*bw*/+ ; *st*/*st*) to males

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of the *bw*; *st* stock in each of the subsequent generations of its inbreeding.

After December of 1950 both mutants were bred as common laboratory stocks until May of 1953 (Slatis, personal communication). At this time bw^{75} was crossed with the related *bw* stock and a single heterozygous female was backcrossed to the parental *bw* strain (since *st* was homozygous in all strains used in this study, it will usually be dropped from subsequent notation). Such backcrosses were repeated for eight generations after which time strains of bw^{75} and *bw* were isolated for use as the foundation stocks of these experiments. These two stocks were, then, closely related, by reason of repeated backcross, common ancestry, and the origin of bw^{75} by mutation in a second chromosome which may be presumed to have been, at the time of irradiation, identical with that of the *bw* strain except in the immediate vicinity of the *bw* locus. The chromosomes of both stocks have been examined by Slatis who has stated that they are free of structural abnormalities (Slatis, personal communication).

The phenotype of the bw^{75} homozygotes (bw^{75}/bw^{75}; *st*/*st*) is a bright red-orange in young flies raised at 25° C. This color darkens somewhat with age and at extreme age may appear a deep red-brown. The heterozygote (bw^{75}/bw; *st*/*st*) is a light orange at this temperature and in newly emerged flies. This, again, darkens with age but is always clearly distinguishable from the bw^{75} homozygotes. The *bw* homozygote (*bw*/*bw*; *st*/*st*) is white. Routine experience has indicated that the eye colors of homozygous bw^{75}; *st* and its heterozygote with *bw*; *st* are temperature dependent and that the distinction between them may not be as clear at lower temperatures. The material of all experiments in this study was maintained in an incubator regulated at 25° C.

The Random Drift Experiments

Two culture series of 107 and 105 bottles respectively were initiated at a gene frequency of 0.50 by introducing 16 bw^{75}/bw flies, 8 male and 8 female, into culture bottles containing about 1 cm. of cornmeal-molasses-agar medium which had been lightly sprinkled with dry baker's yeast. Series I was set up in 35 cc. homeopathic vials with an inside diameter of roughly 2 cm. Series II was cultured in 60 cc. specimen bottles having an inside diameter of about 4 cm. Each successive generation of each bottle was inititated with 16 flies chosen according to the genotypic array in a random sample of 16 flies, 8 male and 8 female, drawn from among the F_1 individuals of the preceding generation of the same culture. Samples were always taken on a particular day after mating, the 12th day in series I and the 14th day in series II. The F_1 individuals appeared on the 10th day in both series. The flies in a bottle to be sampled were etherized, dumped on a paper, and spread out into a line with a brush. The genotypes of the first 8 individuals of each sex to appear in this row characterized the sample.

In order to avoid inbreeding at loci other than brown, an effort was made to reduce or to eliminate the correlation among the parents of any given culture with respect to their origin from among the bottles of the parental generation. Because of the limited number of flies obtainable from the vial cultures of series I after the second day of eclosion it was necessary, for the most part, to use the sample flies themselves as parents of the following generation of the same line. However, the tendency to enforce homozygosis among residual loci was reduced in this series by substituting for one or two flies in a given sample flies of the same phenotype that had been collected as supernumeraries at the time of sampling. In series II the problem could be met more satisfactorily. The eclosion after the twelfth day was sufficiently heavy to make possible the complete separation of sampling and the collection of flies to serve as parents for the next generation. Parental flies were collected on the 12th day from all bottles of the series. Sam-

pling was carried out on the 14th day from among the flies emerging on the 13th day. No flies collected after the 12th day were used as parents in either series and since mating was always carried out on the 14th day the parental flies of each generation of both series were aged two days at the time of mating. Mating was, of course, always according to the genotypic array of the 16 sample flies. All flies used as parents were collected within 12 hours after the cultures had been carefully emptied. Females collected within this period were taken as virgin. More certain criteria of virginity were not feasible because of the large number of parents of nearly uniform age needed to perpetuate a culture series.

The Selection Experiments

To provide data on the mode and magnitude of selection that were independent of the random-drift experiments and would permit a more systematic analysis of the selection parameters than would be possible from the random assortment of population types occurring within the random-drift experiments themselves, a series of experiments was set up designed to present the simplest sort of data on the relative viability and productivity of the several genotypes. Columns 2 and 3 of table 4 present schematically the various types of group mating used to this purpose. Three major categories of population type appear: (I: Comparisons of viability made by examining segregations from group matings between 8 heterozygous females and 8 males of uniform genotype in each case; II: Matings between equal numbers of 2 types of females, 4 each, and 8 individuals of each type of male in all of the 9 possible combinations to test for competitive productivity of females after allowing for possible viability differences among genotypes; III: Reciprocal matings of category II to test for competitive productivity of males.)

These matings were made in 60 cc. specimen jars. The routine of sampling and collection of flies to serve as parents was the same as that for the series II drift experiments with the exception that the sample consisted of 32 flies equally divided between the sexes instead of 16.

In order to distribute equally among the different categories of parent population any heterogeneity in culture conditions such as might be expected among experiments carried out at different times, an effort was made to make 5 replications of each of the 21 population types every time a group of matings was made. The few irregularities that occurred in carrying out this plan were due either to accidental loss of bottles or inadequate numbers of parental flies of appropriate genotype.

Population Cage Experiments on Selection

A number of small population cages were constructed of plexiglas for the purpose of observing competition between the alleles bw^{75} and *bw* under continuous culture and such adverse conditions as might be expected to occur in a confined population facing limited food supply. Selective differences that could easily show no influence on the results of the other experiments, such as longevity, total fecundity, etc. and differences negligible under more favorable conditions of culture might be expected to appear upon prolonged observation of population cages.

The cages had bases measuring roughly 8×13.5 cm. and were about 5 cm. in height. Ventilation was permitted by a 2.2 cm. hole bored in one end and stoppered with a cotton plug that could be renewed periodically. The culture medium was placed on a slant in 35 cc. homeopathic vials which were attached to lucite tubes furnished with pressed cork bushings and set into the long sides of each container, four on each side.

Initially seven populations of 100 heterozygous flies each, 50 male and 50 female, were introduced into as many cages each provided with eight freshly yeasted vials. The females of these parental

groups had been isolated as virgins from the F_1 of a series of matings of bw^{75}/bw^{75} with bw/bw. Before introduction into the cages these flies had been premated in small groups for from one to three days in half-pint bottles containing an abundance of food.

On the 18th day after the initiation of cultures within the cages a single fresh vial was substituted for one of the original vials on each cage. On every third day after this another of the original vials was replaced until all had been removed. After this time the oldest vials remaining were replaced with fresh ones at the same three day interval. Sampling was begun on the 60th day after initiation and was accomplished by counting and classifying the flies that accumulated in a newly attached vial during the first 6 or 8 hours. After classification all flies were returned to the vial which was then re-attached to its cage.

Results

The Population Cage Experiments

Table 1 presents the data from observation of the seven population cages over a period of fifteen weeks. Six observations were made on each cage. At the time of the first sample on the 60th day the frequency of bw^{75} differed strikingly among them. The frequency was in all cases less than 0.50 and ranged from 0.491 to 0.385 with a mean value of $0.446 \pm .013$, significantly below 0.50 ($t = 4.2$, $p < .001$).

There were no significant differences between the estimates of gene frequency based on the male and female components of each sample as is shown by the comparisons appearing in table 2. Accordingly, the frequency in this discussion is always that of the whole sample.

Despite the diversity in frequency among the cages at the time of the first sample, regression coefficients computed for the series of frequency values obtained from all six samples taken from each cage indicated no significant trends during the period of observation in any case. These coefficients together with their standard errors appear in table 1. Three are positive and four negative, and the regression on sample number of the mean values of the frequency is very small and of no significance.

Considering the group of cage experiments as a whole, there is no indication of a difference in relative selective value between the two alleles apart from the fact that the observed frequencies were, for the most part, less than 0.50 throughout the period of observation. The differentiation between cages, which is fairly consistent throughout sampling, must have been due to shifts in frequency during the earliest period. The significance

TABLE 1. *Gene frequency* (bw^{75}) *in 6 successive samples taken at three-week intervals from each of 7 population cages and the mean frequency among all cages at the sample time*

	Cage							
Sample	I	II	III	IV	V	VI	VII	$\bar{q}$
1	.4907	.4598	.4231	.4673	.4527	.3853	.4420	.4458
2	.4757	.4044	.3985	.4156	.4377	.3833	.4244	.4194
3	.4613	.4405	.4459	.4545	.4638	.3944	.4263	.4410
4	.4987	.4655	.3750	.4296	.5209	.3941	.4459	.4471
5	.5282	.4574	.4087	.3706	.4665	.3425	.3913	.4236
6	.5138	.4326	.4167	.4103	.4771	.4019	.3842	.4338
b_{yx}	+.0089	+.0043	−.0021	−.0127	+.0079	−.0011	−.0105	−.0012
S.E.	.0048	.0057	.0055	.0211	.0068	.0056	.0044	.0030
t	1.85	.75	.38	.60	1.16	.20	2.39	.40
p	.2–.1	.5–.4	.8–.7	.6–.5	.3–.2	.9–.8	.1–.05	≃.7

TABLE 2. *Comparison of male and female gene frequency estimates in cage samples. Standard errors computed as* $\sqrt{q_t(1-q_t)(1/N_m + 1/N_f)}$ *where* q_t *is the frequency in the sexes combined.*

Cage No.	Sample No.	N ♂	N ♀	q ♂	q ♀	q ♂ − q ♀	S.E.	t
I	1	863	744	.489	.493	−.004	.025	.2
	2	89	137	.500	.460	+.040	.068	.6
	3	211	151	.453	.474	−.021	.053	.4
	4	206	176	.507	.489	+.019	.051	.4
	5	108	105	.472	.586	−.114	.068	1.7
	6	121	132	.484	.542	−.058	.063	.9
II	1	136	125	.445	.476	−.031	.062	.5
	2	86	139	.436	.385	+.051	.067	.8
	3	153	158	.464	.418	+.046	.056	.8
	4	181	109	.472	.454	+.018	.061	.3
	5	92	96	.446	.469	−.023	.073	.3
	6	92	101	.451	.416	+.035	.071	.5
III	1	132	102	.409	.441	−.032	.065	.5
	2	112	159	.393	.403	−.010	.062	.2
	3	155	104	.445	.447	−.002	.063	.3
	4	121	143	.401	.353	+.048	.060	.8
	5	110	109	.400	.417	−.017	.066	.3
	6	84	84	.446	.387	+.059	.076	.8
IV	1	130	115	.477	.457	+.020	.064	.3
	2	81	162	.383	.432	−.049	.067	.7
	3	238	169	.450	.462	−.012	.050	.2
	4	195	146	.464	.384	+.080	.054	1.5
	5	99	98	.369	.372	−.003	.069	.0
	6	85	99	.453	.374	+.079	.073	1.1
V	1	121	143	.471	.437	+.034	.062	.5
	2	111	138	.396	.464	−.068	.063	1.1
	3	173	131	.448	.485	−.037	.058	.6
	4	123	92	.500	.549	−.049	.069	.7
	5	81	83	.469	.464	+.005	.078	.0
	6	116	102	.444	.515	−.071	.068	1.0
VI	1	102	129	.402	.372	+.030	.065	.5
	2	106	194	.426	.361	+.065	.059	1.1
	3	174	186	.394	.395	−.001	.052	.0
	4	141	81	.362	.451	−.089	.068	1.3
	5	102	79	.328	.361	−.033	.071	.5
	6	73	141	.370	.418	−.048	.071	.7
VII	1	162	157	.426	.459	−.033	.056	.6
	2	97	141	.407	.436	−.029	.065	.4
	3	170	115	.409	.452	−.043	.060	.7
	4	141	81	.440	.457	−.017	.069	.2
	5	79	82	.399	.384	+.015	.077	.2
	6	64	139	.383	.385	−.002	.074	.0

of the mean shift suggests a systematic influence effective only during the early history of the cages prior to the time of the first count.

A striking variation in the sex ratio within samples was apparent. Tests of significance of the departure from the expected proportion were applied to each sample from all cages and appear in table 3. Standard errors were based on a

theoretical value of 0.50. Of a total of 42 cases, 17 (roughly 40%) of the differences have t-values greater than 2.6 ($p = 0.01$) and 9 (about 21%) show t-values greater than 3.3 ($p = 0.001$). A t-test on the total numbers from all cages produced a value of 1.274 ($p = 0.30 - 0.20$) in support of the hypothetical fre-

TABLE 3. *Comparison of the observed proportion of males in population cage samples with a theoretical value of .50. Standard errors are based on the expected proportion.*

Cage No.	Sample No.	N	Males	O−C	S.E.	t
I	1	1607	.537	+.037	.013	3.0**
	2	226	.394	−.106	.033	3.2**
	3	362	.583	+.083	.026	3.2**
	4	382	.539	+.040	.026	1.5
	5	213	.507	+.007	.034	.2
	6	253	.478	−.022	.031	.7
II	1	261	.521	+.021	.031	.7
	2	225	.382	−.118	.033	3.6***
	3	311	.492	−.008	.028	.3
	4	290	.624	+.124	.029	4.3***
	5	188	.489	−.011	.037	.3
	6	193	.477	−.023	.036	.6
III	1	234	.564	+.064	.033	1.9*
	2	271	.413	−.087	.030	2.9**
	3	259	.599	+.099	.031	3.2**
	4	264	.458	−.042	.037	1.1
	5	219	.502	+.002	.034	.1
	6	168	.500	.000	.039	.0
IV	1	245	.531	+.031	.032	1.0
	2	243	.333	−.167	.033	5.1***
	3	407	.585	+.085	.025	3.4***
	4	341	.572	+.072	.027	2.7**
	5	197	.503	+.003	.036	.1
	6	184	.462	−.038	.037	1.0
V	1	264	.458	−.042	.031	1.4
	2	249	.446	−.054	.032	1.7
	3	304	.569	+.069	.029	2.4*
	4	215	.572	+.072	.034	2.1*
	5	164	.494	−.006	.039	.2
	6	218	.532	+.032	.034	1.0
VI	1	231	.442	−.058	.033	1.8
	2	300	.353	−.147	.029	5.1***
	3	360	.483	−.017	.026	.6
	4	222	.635	+.135	.034	4.0***
	5	181	.564	+.064	.037	1.7
	6	214	.341	−.159	.034	4.7***
VII	1	319	.508	+.008	.028	.3
	2	238	.408	−.092	.032	2.9**
	3	285	.597	+.097	.030	3.2**
	4	222	.635	+.135	.034	4.0***
	5	161	.491	−.009	.039	.2
	6	203	.315	−.185	.035	5.3***
Total		11893	.506	+.006	.005	1.2

quency of 0.50 used in calculating standard errors. The signs of the deviations show that they were as nearly equally distributed about the theoretical mean as possible both within the group showing significance beyond the 1% level and among all cases.

This highly significant and wholly unsystematic disturbance may have been due to a periodicity in the rate of emergence reflecting the three day interval of vial exchange. In these stocks routine observations have shown that males develop more rapidly than females. The limited capacity of a single culture vial would probably restrict the period of effective oviposition to within a few hours of the substitution of a fresh vial for an old. If this were the case and if, as seems likely, the longevity of adults was very much decreased by, for example, the scarcity of food or the accumulation of toxic substances, the fluctuation in sex ratio observable throughout the eclosion period of any single bottle culture would affect the sex ratio of the entire cage population.

No good way of estimating the size of the populations within these cages has been found. A total count was made only in the case of the first sampling of cage number 1. Because only those flies that could be induced to leave the cage and enter fresh bottles could be counted, this census cannot be considered complete. A total of 1,607 flies was collected, however, which indicates that cages of these specifications can maintain very large populations. No answer can be made to the important question of how much fluctuation in population size occurred during the 23 weeks these cages were maintained.

The Selection Experiments

The results of the selection experiments appear in table 4. The type of mating, the observed and expected segregations, their differences, standard errors and t-values are presented. Standard errors were based on the distribution of the observed proportion of a single genotype among all bottles of a given population type and have been applied only to the cases in which there were only two genotypes among the offspring.

The offspring of matings within group I which was designed to test for differences in viability among the several genotypes show no significant departures from expectation. It is therefore inferred that the results of the productivity mating types can be interpreted assuming no differential viability.

It is evident that with the exception of population types II C a, III C a and III C c no significant departures from expectation obtained in the productivity series. In these three cases, however, the deviations were highly significant with t-values of 3.0, 3.3 and 3.3 respectively.

These results taken at face value indicate that there is no difference in the productivity of bw^{75}/bw^{75} and bw^{75}/bw females or of bw^{75}/bw and bw/bw females in "competition" for any single type of male. (Competition is not meant to imply any preconception with regard to the factors operative in this situation.) Similarly, when the competing individuals in these categories are males there are no apparent differences between the alternatives in frequency or effectiveness of insemination of any single female type. However, when bw^{75}/bw^{75} and bw/bw females compete the latter are the more productive when the male type is bw^{75}/bw^{75} but no difference is apparent when the male type is either bw^{75}/bw or bw/bw. When the productivities of bw^{75}/bw^{75} and bw/bw males are compared, the bw^{75}/bw^{75} males leave more offspring when the females are either bw^{75}/bw^{75} or bw/bw but show no significant difference from bw/bw when competing for bw^{75}/bw females.

If these differences be accepted as valid evidence of the situation their expression is obviously highly dependent upon the specific pair of genotypes of the competing individuals as well as on the genotype presented by the opposite sex. No systematic explanation suggests itself upon

TABLE 4. *Observed segregations in selection experiments.* Standard errors are based on the variance of the observed distributions of single F_1 phenotypes and are applied only to two-class segregations.

Mating type	Parental population genotype: Females $\frac{bw^{75}}{bw^{75}}$	Females $\frac{bw^{75}}{bw}$	Females $\frac{bw}{bw}$	Males $\frac{bw^{75}}{bw^{75}}$	Males $\frac{bw^{75}}{bw}$	Males $\frac{bw}{bw}$	N	Observed segregation $\frac{bw^{75}}{bw^{75}}$	Observed segregation $\frac{bw^{75}}{bw}$	Observed segregation $\frac{bw}{bw}$	Observed − Expected $\frac{bw^{75}}{bw^{75}}$	Observed − Expected $\frac{bw^{75}}{bw}$	Observed − Expected $\frac{bw}{bw}$	S.E.	t
I-A		8		8			59	.513	.487		+.013	−.013		.009	1.4
I-B		8			8		58	.245	.510	.245	−.005	+.010	−.005		
I-C		8				8	60		.504	.496		+.004	−.004	.013	0.3
II-A-a		4	4	8			58	.230	.770		−.020	+.020		.011	1.8
II-A-b		4	4		8		59	.126	.499	.375	+.001	−.001	.000		
II-A-c		4	4			8	58		.251	.749		+.001	−.001	.012	.0
II-B-a	4	4		8			58	.733	.267		−.017	+.017		.015	1.1
II-B-b	4	4			8		58	.363	.497	.140	−.012	−.003	+.015		
II-B-c	4	4				8	55		.739	.261		−.011	+.011	.015	.7
II-C-a	4		4	8			54	.436	.564		−.064	+.064		.021	3.0*
							(80)	(.541)	(.459)		(+.041)	(−.041)		(.022)	(1.9)
II-C-b	4		4		8		59	.231	.508	.261	−.019	+.008	+.011		
II-C-c	4		4			8	60		.501	.499		+.001	−.001	.020	.0
							(81)		(.493)	(.507)		(−.007)	(+.007)	(.018)	(0.4)
III-A-a	8				4	4	59	.265	.735		+.015	−.015		.015	1.0
III-A-b		8			4	4	59	.131	.485	.384	+.005	−.015	+.009		
III-A-c			8		4	4	60		.263	.737		+.013	−.013	.015	0.9
III-B-a	8			4	4		60	.740	.260		−.010	+.010		.017	0.6
III-B-b		8		4	4		49	.375	.499	.126	.000	−.001	+.001		
III-B-c			8	4	4		59		.722	.278		+.028	−.028	.016	1.8
III-C-a	8			4		4	58	.578	.422		+.078	−.078		.023	3.4*
							(80)	(.498)	(.502)		(−.002)	(+.002)		(.019)	(0.1)
III-C-b		8		4		4	55	.244	.510	.246	−.006	+.010	−.004		
III-C-c			8	4		4	60		.596	.404		+.096	−.096	.030	3.2*
							(82)		(.496)	(.504)		(−.004)	(+.004)	(.032)	(0.2)

examination of these data. There is no self-determined consistency in the behavior of any genotype.

To furnish further evidence on the segregations in categories II C and III C that would either lend credence to or discredit the initial observations, an additional number of replicates, somewhat larger than that in the initial series, was made of categories II C a, II C c, III C a, and III C c. The values obtaining from these observations appear in table 4 in parenthesis below the initially determined values. No evidence of significant deviations appears among them. Although it seems unlikely that the cases of significance in the first set of observations were entirely fortuitous, the fact that no more than five replicates of any population type were made at any given time substantially rules out the possibility that the groups in which significant deviations were observed differed systematically from other groups in environmental conditions. The factors responsible for these shifts remain obscure.

To check against possible heterogeneity among groups of replicates made at different times, an analysis of variance comparing the variability within and between groups of the same population type was carried out. This test is not strictly applicable to data of a binomial character because of the relationship between the mean (np) and variance (npq) but should serve as a fair test in this case since no comparisons have been made between population categories having different theoretical means and these are in no case higher than 0.75 or lower than 0.25 at which values skewness and kurtosis are negligible for such values of n as occur in these cases.

The results of this analysis are presented in table 5. None of the F values was found to be significant beyond the 1% level and only two had probabilities between 0.01 and 0.05. In one of the cases showing probable significance, the variance between groups was smaller than that within groups, which suggests no heterogeneity. The total variances cannot have been appreciably affected by variation in environmental conditions during the period of observations.

The data from the additional replicates

TABLE 5. *Analysis of variance comparing variability within and between groups of replicates in selection experiments.* All values on an absolute scale.

Mating type		D.F.	Mean square	F	Mating type		D.F.	Mean square	F
I-A	within	48	9.1	1.8	I-C	within	47	5.0	1.3
	between	11	16.0			between	11	3.8	
II-A-a	within	46	7.2	1.4	II-A-c	within	46	8.2	1.0
	between	11	5.2			between	11	8.4	
II-B-a	within	46	11.4	1.7	II-B-c	within	44	12.7	1.2
	between	11	19.8			between	10	15.4	
II-C-a	within	43	25.9	1.1	II-C-c	within	48	24.8	1.1
	between	10	23.0			between	11	28.2	
III-A-a	within	48	14.8	2.8*	III-A-c	within	48	14.6	1.0
	between	11	5.2			between	11	14.9	
III-B-a	within	48	15.0	2.3*	III-B-c	within	47	14.2	1.5
	between	11	34.5			between	11	21.6	
III-C-a	within	46	34.3	1.4	III-C-c	within	48	51.0	1.0
	between	11	24.7			between	11	52.2	

TABLE 6. *Comparison of total variance between appropriate categories in productivity matings of selection experiments.* Variance ratio interpreted on F table of Snedecor. All values are on an absolute scale.

Mating type	d.f.	Mean square	F	Mating type	d.f.	Mean square	F
II-A-a	57	6.8	1.2	III-A-a	58	13.0	1.1
II-A-c	57	8.3		III-A-c	59	14.7	
II-B-a	57	13.1	1.0	III-B-a	59	18.7	1.2
II-B-c	54	13.2		III-B-c	58	15.6	
II-C-a	53	25.4	1.0	III-C-a	57	32.4	1.6*
II-C-c	59	25.4		III-C-c	59	51.2	
II-C-a′	79	38.8	1.4	III-C-a′	79	28.8	1.6*
II-C-c′	80	28.3		III-C-c′	81	45.1	
		(Comparison between categories II and III)					
II-A-a	57	6.8	1.9**	II-A-c	57	8.3	1.8*
III-A-a	59	13.0		III-A-c	59	14.7	
II-B-a	57	13.1	1.4	II-B-c	54	13.2	1.2
III-B-a	59	18.7		III-B-c	58	15.6	
II-C-a	53	25.4	1.3	II-C-c	59	25.4	2.0**
III-C-a	57	32.4		III-C-c	59	51.2	
II-C-a′	79	38.8	1.3	II-C-c′	80	28.3	1.6*
III-C-a′	79	28.8		III-C-c′	81	45.1	
		(Comparison between categories A and B)					
II-A-a	57	6.8	1.9**	III-A-a	59	13.0	1.4
II-B-a	57	13.1		III-B-a	59	18.7	
II-A-c	57	8.3	1.6*	III-A-c	59	14.7	1.1
II-B-c	54	13.2		III-B-c	58	15.6	

of the population types in categories II C and III C made outside the body of the main experiment could not be included in this analysis since they were collected over a relatively short period of time and were from series of replicates that were grouped unequally and of larger size than the standard of five set for the initial study. The lack of evidence of heterogeneity between groups in the initial experiment indicates that this difference in treatment is not likely to have affected to an appreciable extent the outcome of the later study.

The results of population types presenting three classes of F_1 progeny show only small and unsystematic deviations from expectation. In the absence of clear evidence of selection from the other categories, no analysis of these types seems necessary.

It is of interest to compare the variability of the observed phenotypic frequencies among the offspring of certain comparable population types both within and between male and female productivity series. The appropriate comparisons are presented in table 6. A rough test of the significance of the variance differences is given by the variance ratio interpreted as F.

Among the comparisons of a and c categories (bw^{75}/bw^{75} vs bw/bw as the one-genotype parental group), the ratios are in all but one case clearly not significantly

different from unity. The ratio is probably significant, $p = 0.05 - 0.01$, in the comparison of III C a′ with III C c′, and aproaches significance at the 5% level in the corresponding case in the initial survey. But, since there is no consistency in the sign of the variance differences in the other comparisons of this type within the male productivity matings, there is not sufficient evidence to assign this affect to the productivity variation of a particular genotype.

With regard to the comparisons between categories II and III (reciprocal mating types), the variances of the observed segregations are in all but one case greater in male productivity population tests than in female. In the comparison of classes II A c and III A c the variance ratio is significant with a probability between 0.05 and 0.01. Further, within the initial series of matings, the comparison of the variance of II C c with III C c yields an F-value significant beyond the 1% level. The variances of the segregations in the groups of the subsidiary study are closely comparable to those of the initial series in the cases of II C c′, III C a′ and III C c′ but are not in such good agreement in the case of II C a′. The comparison of II C a′ with III C a′ is the only case in which the female productivity variance was found to be higher than the male in comparable categories.

The most interesting differences appear in the comparisons between A and B categories (tests of $bw/bw : bw^{75}/bw$ competitions vs those of the $bw^{75}/bw^{75} : bw^{75}/bw$ competitions). Both such comparisons show significant differences in the case of females. The ratio of the variances of II A a and II B a is 1.913 which is significant at about the 1% level, that of the variances of II A c and II B c is 1.602 and significant at about the 5% level. Neither of the comparisons within male categories is significant; although the III B categories have higher variances than the III A categories in absolute terms, the differences are slight.

The difference in variance between the A and B categories in females is associated with the presence of the bw^{75}/bw^{75} genotype. These data suggest that this genotype is considerably more variable in its productivity than either of the other female genotypes, a fact that may be of some importance to the discussion in a later section.

It should be pointed out that the observed segregation variances are not directly related to the variation in productivity of the competing parents in the cases of the four C categories. In all such cases at least one of the segregation genotypes is produced by both of the parental types, male or female, under consideration. The variances of the segregations in the C categories would, therefore, be expected to be larger for this reason alone. Since the segregations in the C categories have theoretical means of 0.50 while those of A and B categories with two-class segregations have theoretical means of 0.25 or 0.75, the variances of C categories would be expected to be about 1/3 again as large as those in either A or B in samples of equal size, a variance ratio of 1.33. Tests of significance would accordingly be inappropriate in these cases. All ratios involving C categories were very much greater than 1.33, ranging from 1.737 to 3.717.

Random Drift Experiments

Selection

To get evidence on the question of selection from within the body of the experimental data of the random drift experiments, tests of significance were applied in each generation to the mean differences in frequency between bottles at the time of mating and at the time of sampling. Standard errors were calculated from the variances of the observed distributions of these differences among bottles. The mean change in frequency may be represented by $\overline{\Delta q}$. These data appear in tables 7 and 8 for random drift series I and II respectively, and are presented graphically in figure 1.

TABLE 7. *Series I. Gene frequency changes by generation; q_t = total frequency including previously fixed cultures, q_m = frequency among unfixed cultures at the time of mating, q_e = frequency among unfixed and newly fixed cultures at the time of sampling.* Standard errors are computed from the observed variance of Δq among bottles in each generation.

Gen.	q_t	N	q_m	q_e	$q_e - q_m$ ($\overline{\Delta q}$)	S.E.$_{\overline{\Delta q}}$	t
0	.500	107	.500	.506	+.006	.008	.8
1	.506	107	.506	.494	−.012	.011	1.1
2	.494	107	.494	.495	+.001	.012	.1
3	.495	107	.495	.490	−.005	.011	.5
4	.490	106	.486	.489	+.003	.010	.3
5	.494	105	.484	.459	−.025	.010	2.5*
6	.469	103	.458	.471	+.013	.010	1.3
7	.482	101	.481	.479	−.002	.010	.2
8	.480	97	.478	.473	−.005	.012	.4
9	.476	96	.468	.477	+.009	.012	.8
10	.484	92	.476	.486	+.010	.012	.8
11	.492	86	.496	.504	+.008	.010	.8
12	.499	78	.466	.462	−.004	.011	.4
13	.495	77	.455	.452	−.003	.012	.3
14	.493	71	.448	.458	+.010	.013	.8
15	.500	66	.463	.471	+.008	.011	.7
16	.505	59	.493	.466	−.027	.011	2.5*
17	.490	55	.481	.463	−.018	.012	1.5
18	.481	52	.451	.432	−.019	.010	1.9
19	.472	—	(.432)	—	—	—	—
0–19	.4899	1672	.4796	.4778	−.0018	.0025	.72
1–19	.4894	1565	.4782	.4759	−.0023	.0027	.85

The data from series I contain little evidence of selection. The t-values indicate decreases in the frequency of *bw*^{75} between generations 5 and 6 and 16 and 17 that are significant with probabilities of 0.009 and 0.02 respectively. ("generation" is used here to refer to the parental population alone.) Grouping all shifts in frequency between generations, the value of $\overline{\Delta q}$ is not significant, $p = 0.48$. However, since the deviations between generations 0 and 1 were not subject to the influence of variations in productivity they should perhaps be omitted in computing the overall value of $\overline{\Delta q}$. Omitting these deviations the value of $\overline{\Delta q}$ is only slightly different and of no significance, $p = 0.4 - 0.3$. There is no evidence of any persistent trend in the sign of $\overline{\Delta q}$ in the course of the experiment and the array of t-values does not suggest any heterogeneity between data collected at different times.

Frequencies of *bw*^{75} involve, in the case of mating frequencies, only unfixed cultures, and in the case of collection frequencies, only unfixed and newly fixed populations. It is of interest to note the trend in total gene frequency change considering in each generation previously fixed as well as newly fixed and unfixed classes. These frequencies for both series appear under q_t in tables 7 and 8 and in figure 1.

No systematic trend is apparent in the case of series I. The mean total frequency over all generations is 0.4899 and the ratio of fixation at the two extremes is 30 (*bw*) : 28 (*bw*^{75}), approaching equality as would be expected in the absence of selection.

In the case of series II (table 8 and fig. 1) there was a consistent increase in the frequency of *bw*^{75} during the first 4 generations. The values of $\overline{\Delta q}$ between generations 2 & 3 and 3 & 4 are both

positive and significant at about the 2% and 1% levels respectively. The value of $\overline{\Delta q}$ between generations 17 & 18 is significant at about the 2% level and that taken over the entire sequence of generations is significant at the 5% level. However, omitting the changes between generations 0 and 1 for the same reason as was given in connection with series I, the overall value is somewhat less and of no significance, $t = 1.83, p = 0.10 - 0.05$. The overall shift must be regarded as of doubtful significance.

The total frequency in each generation including previously fixed classes rises during the first four generations to a value of 0.5711 (fig. 1). This frequency subsequently fluctuates somewhat but remains fairly stable. The mean total frequency over the last 16 generations is 0.5674. The gradual drop in frequency in the array of unfixed and newly fixed populations after the 4th generation is due wholly to a higher rate of fixation for *bw*^{75} than for *bw* in this series rather than to consistent negative selection over this period. The net change in gene frequency attributable to selection between generations 5 and 19 is, in fact, positive (+.0083). The ratio of fixation at the two extremes is 13 (*bw*) ; 30 (*bw*^{75}), $t = 3.08$, $p = 0.01 - 0.001$. This asymmetry is clearly related to the steady increase in the value of $q_{bw^{75}}$ over the first four generations.

When the observed sample frequencies of each series are classified according to the mating frequency rather than by generation and the means of the resulting recipient class distributions are compared with their respective theoretical values, no evidence of selection is found in either case. Table 9 presents these data for series I and II. The standard errors

TABLE 8. *Series II. Gene frequency changes by generation; q_t = total frequency including previously fixed cultures, q_m = frequency among unfixed cultures at the time of mating, q_e = frequency among unfixed and newly fixed cultures at the time of sampling.* Standard errors are computed from the observed variance of Δq among bottles in each generation.

Gen.	q_t	N	q_m	q_e	$q_e - q_m$ ($\overline{\Delta q}$)	S.E.$_{\overline{\Delta q}}$	t
0	.500	105	.500	.509	+.009	.009	1.0
1	.509	105	.509	.522	+.013	.010	1.3
2	.522	105	.522	.546	+.024	.010	2.4*
3	.546	105	.546	.571	+.025	.009	2.8**
4	.571	104†	.570	.569	−.001	.010	.1
5	.569	103	.564	.575	+.011	.010	1.1
6	.579	102	.571	.560	−.011	.008	1.4
7	.569	100	.552	.547	−.005	.010	.5
8	.565	95	.534	.535	+.001	.010	.1
9	.566	93	.536	.541	+.005	.010	.5
10	.570	91	.542	.547	+.005	.009	.6
11	.575	90	.542	.539	−.003	.010	.3
12	.572	88	.529	.523	−.006	.011	.5
13	.568	84	.525	.522	−.003	.011	.3
14	.566	79	.492	.477	−.015	.010	1.5
15	.555	76	.483	.486	+.003	.010	.3
16	.557	74	.473	.469	−.004	.010	.4
17	.555	69	.488	.511	+.023	.010	2.3*
18	.570	61	.480	.484	+.004	.011	.4
19	.572	—	(.484)	—	—	—	—
0–19	.5578	1729	.5273	.5317	+.0044	.0022	2.00*
1–19	.5608	1624	.5290	.5332	+.0042	.0023	1.83

† Accidental loss.

were computed as $\sqrt{\sigma_o^2/N}$. Although a standard error of this sort takes no account of the expected asymmetry of the distributions at the extremes of frequency, examination of the array of t-values together with the signs of the differences between observed and theoretical means indicates that there can be little doubt as to the points of significance. In each series only three of the differences are significant, none beyond the 1% level. There are no consistent trends in sign in either case and it can therefore be safely assumed that any changes in gene frequency attributable to selection in either series are wholly negligible in this context.

The Variance of Recipient Class Gene Frequencies

In the absence of appreciable selection and assuming random mating and Poisson variability in productivity of parents (cf. Crow, 1955) the variances of the recipient class frequencies grouped about each of the 31 donor class frequencies as means should be binomial in character and equal to $q(1-q)/2N_e$, where $2N_e$ equals the effective sample size in terms

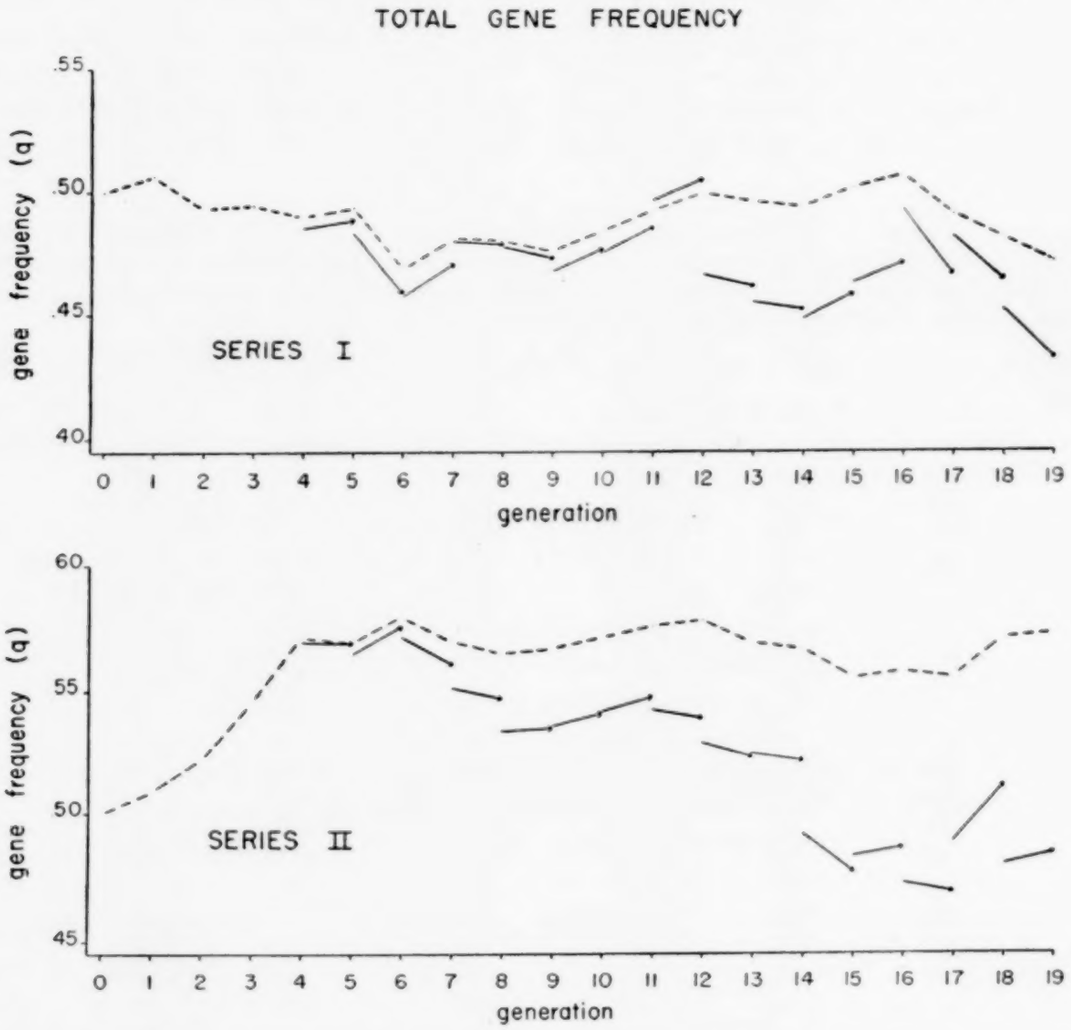

FIG. 1. Total gene frequency by generation for series I and II. Frequency including previously fixed as well as newly fixed cultures is indicated by the dashed line. The arrows represent the changes in gene frequency between generations among unfixed bottles. The break in this line at any generation is brought about by frequency changes due to unequal fixation for the two alleles.

TABLE 9. *Series I and II. Comparison of observed with theoretical recipient class means;* $\bar{q}_{th} = 1/32$ *class value.* Standard errors are based on the variance of q_o about q_{th}.

Class value	Series I					Series II				
	n	$\bar{q}_o$	$\bar{q}_o - \bar{q}_{th}$	S.E.	t	n	$\bar{q}_o$	$\bar{q}_o - \bar{q}_{th}$	S.E.	t
1	25	.018	−.014	.006	2.3*	18	.040	+.009	.011	.8
2	25	.081	+.019	.012	1.6	24	.066	+.004	.008	.5
3	43	.088	−.006	.010	.6	20	.072	−.022	.013	1.7
4	38	.119	−.006	.012	.5	22	.124	−.001	.015	.1
5	31	.137	−.019	.013	1.5	20	.158	+.002	.017	.1
6	46	.166	−.021	.011	1.9	29	.193	+.005	.018	.3
7	45	.251	+.032	.018	1.8	44	.212	−.006	.011	.5
8	47	.242	−.008	.011	.7	38	.273	+.023	.013	1.8
9	64	.284	+.003	.013	.2	46	.295	+.014	.014	1.0
10	52	.311	+.002	.016	.1	53	.315	+.003	.014	.2
11	60	.338	−.006	.015	.4	55	.339	−.005	.012	.4
12	69	.382	−.007	.011	.6	61	.385	+.010	.013	.8
13	79	.415	+.009	.014	.6	76	.411	+.005	.012	.4
14	78	.435	−.002	.012	.2	90	.447	+.009	.011	.8
15	95	.478	+.009	.012	.8	94	.493	+.024	.010	2.4*
16	109	.494	−.006	.011	.5	89	.511	+.011	.012	.9
17	83	.540	+.008	.013	.6	102	.533	+.002	.010	.2
18	86	.537	−.025	.014	1.8	88	.557	−.006	.011	.5
19	66	.565	−.029	.014	2.1*	93	.580	−.014	.011	1.3
20	63	.622	−.003	.013	.2	66	.612	−.013	.012	1.1
21	46	.649	−.007	.020	.3	52	.665	+.009	.015	.6
22	45	.687	−.001	.017	.1	59	.702	+.014	.014	1.0
23	35	.674	−.045	.023	2.0*	55	.722	+.003	.014	.2
24	39	.776	+.026	.016	1.6	62	.749	−.002	.011	.2
25	33	.771	−.010	.019	.5	33	.791	+.010	.017	.6
26	35	.835	+.022	.017	1.3	45	.811	−.001	.013	.1
27	27	.854	+.011	.015	.7	38	.849	+.005	.013	.4
28	32	.869	−.006	.014	.4	44	.893	+.018	.009	2.0*
29	22	.915	+.009	.016	.6	39	.911	+.005	.009	.6
30	25	.929	−.009	.015	.6	42	.936	−.002	.006	.3
31	22	.977	+.009	.023	.4	27	.982	+.013	.006	2.2*

of genes, theoretically = 32 in the present case. Estimates of $2N_e$ can be made for each class of mating frequency as $q(1-q)/\sigma_o^2$, where σ_o^2 is the observed recipient class variance.

The magnitude of the effect of random sampling in bringing about gene frequency changes is inversely proportional to $2N_e$, and directly proportional to $q(1-q)$. Under circumstances in which the effective population number fluctuates in time an overall estimate of $2N_e$ may be made as the harmonic mean of the values at successive intervals (Wright 1939). In the present case, in which each class estimate is itself the harmonic mean of the values by generation, the 31 class estimates may be combined in this way if it is assumed that $2N_e$ is itself independent of q:

$$2\bar{N}_e = N/\Sigma[1/2N_e]$$

Values of 17.86 and 22.99 were obtained for series I and II respectively.

These estimates of the average value of $2N_e$ for each of the two series can be used as a means of judging the homogeneity of the array of observed variances with respect to this parameter. Tables 10 and 11 present a comparison of these variances with theoretical values computed for each class as $q(1-q)/2N_e$. Standard errors are based on the theoretical variances and take into account the degree of kurtosis expected at each

value of q:

$$S.E. = \sqrt{q(1-q)[4\overline{N}_e q(1-q) + 1 - 6q(1-q)]/n(2\overline{N}_e)^3}$$

(adapted from Fisher, 1925), where $\overline{N}_e$ = the effective sample number and n = the number of samples.

The data of series I (table 10) are markedly heterogeneous; 6 of the 31 t-values are greater than 1.96. In the case of series II there is no evidence of significant discrepancy (table 11). Graphical comparisons (figs. 2 and 3) indicate that the data of series I are sensibly in poor accord with expectation as contrasted with series II. In particular, the observed distribution is decidedly skewed, indicating a tendency toward smaller values of $1/2N_e$ at lower values of q. It may be noted that there is a suggestion of positive skewness in the graph of the recipient class variance data of series II. Equations of the linear regression of class estimates of $1/2N_e$ on q were computed for both series, weighting each value by the total frequency in the recipient class distribution upon which each is based:

$$\text{I:}\quad 1/2N_e = \sigma^2_{\delta q}/q(1-q) = .0451 + .0228q, \quad S.E._b = .0014, \quad t = 16.3$$

$$\text{II:}\quad 1/2N_e = \sigma^2_{\delta q}/q(1-q) = .0427 - .0020q, \quad S.E._b = .0009, \quad t = 2.2$$

TABLE 10. *Series I. Comparison of observed recipient class variances with theoretical values based on a common estimate of $2N_e = 18$.* Class value = $32q_{th}$. All values are presented on a relative scale.

Class value	Obs.	Th.	(Obs. − Th.)	S.E.	t
1	.0010	.0017	−.0007	.0006	1.2
2	.0038	.0033	+.0005	.0011	.5
3	.0043	.0047	−.0004	.0011	.4
4	.0058	.0061	−.0003	.0015	.2
5	.0051	.0073	−.0022	.0019	1.2
6	.0053	.0085	−.0032	.0018	1.8
7	.0139	.0095	+.0044	.0020	2.2*
8	.0059	.0104	−.0045	.0021	2.1*
9	.0103	.0112	−.0009	.0020	.5
10	.0126	.0119	+.0007	.0023	.3
11	.0132	.0125	+.0007	.0022	.3
12	.0079	.0130	−.0051	.0022	2.3*
13	.0160	.0134	+.0026	.0021	1.2
14	.0112	.0137	−.0025	.0021	1.2
15	.0146	.0138	+.0008	.0020	.4
16	.0131	.0139	−.0008	.0018	.4
17	.0150	.0138	+.0012	.0021	.6
18	.0164	.0137	+.0027	.0020	1.4
19	.0121	.0134	−.0013	.0023	.6
20	.0109	.0130	−.0021	.0023	.9
21	.0176	.0125	+.0051	.0026	2.0*
22	.0137	.0119	+.0018	.0025	.7
23	.0186	.0112	+.0074	.0026	2.8**
24	.0102	.0104	−.0002	.0023	.1
25	.0113	.0095	+.0018	.0023	.8
26	.0097	.0085	+.0012	.0020	.6
27	.0063	.0073	−.0010	.0020	.5
28	.0061	.0061	+.0000	.0016	.0
29	.0054	.0047	+.0007	.0015	.5
30	.0054	.0033	+.0021	.0011	1.9*
31	.0012	.0017	−.0005	.0007	.7

TABLE 11. *Series II. Comparison of observed recipient class variances with theoretical values based on a common estimate of $2N_e = 23$.* Class value = $32q_{th}$. All values are presented on a relative scale.

Class value	Obs.	Th.	(Obs. − Th.)	S.E.	t
1	.0022	.0013	+.0009	.0006	1.5
2	.0017	.0025	−.0008	.0008	1.0
3	.0031	.0037	−.0006	.0012	.5
4	.0048	.0048	−.0000	.0015	.0
5	.0059	.0057	+.0002	.0018	.1
6	.0097	.0066	+.0031	.0018	1.7
7	.0056	.0074	−.0018	.0016	1.1
8	.0062	.0082	−.0020	.0019	1.0
9	.0086	.0088	−.0002	.0018	.1
10	.0106	.0093	+.0013	.0018	.7
11	.0074	.0098	−.0024	.0018	1.3
12	.0099	.0102	−.0003	.0018	.2
13	.0115	.0105	+.0010	.0017	.5
14	.0114	.0107	+.0007	.0016	.4
15	.0095	.0108	−.0013	.0015	.9
16	.0133	.0109	+.0024	.0016	1.5
17	.0094	.0108	−.0014	.0015	.9
18	.0099	.0107	−.0012	.0016	.8
19	.0120	.0105	+.0015	.0015	1.0
20	.0100	.0102	−.0002	.0017	.1
21	.0112	.0098	+.0014	.0019	.7
22	.0108	.0093	+.0015	.0017	.9
23	.0103	.0088	+.0015	.0017	.9
24	.0080	.0082	−.0002	.0015	.1
25	.0090	.0074	+.0016	.0018	.9
26	.0071	.0066	+.0005	.0014	.4
27	.0066	.0057	+.0009	.0013	.7
28	.0034	.0048	−.0014	.0010	1.4
29	.0034	.0037	−.0003	.0009	.3
30	.0017	.0025	−.0008	.0006	1.3
31	.0010	.0013	−.0003	.0005	.6

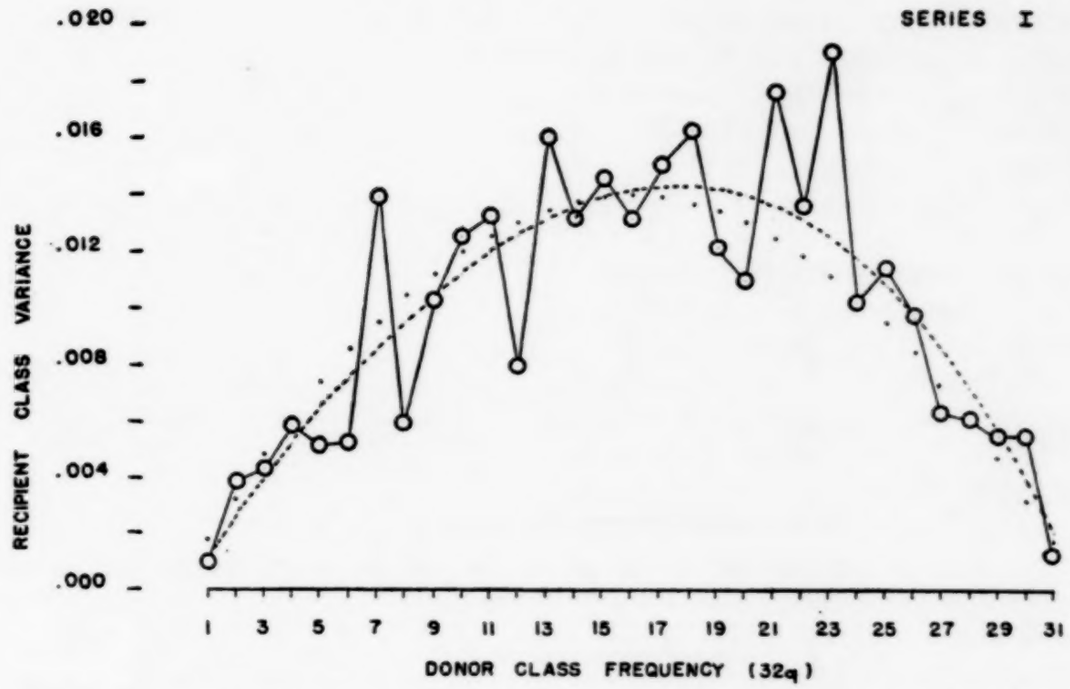

FIG. 2. Comparison of the observed recipient class variances of series I with theoretical values based upon the mean estimates of $1/2N_e$ (dots) and with values based upon the regression equation representing the observed dependence of $1/2N_e$ on q (dashed line).

The effect is highly significant in the case of series I (fig. 4). Considering the graphical presentation of the series II data (fig. 5) no significance can be ascribed to the slight negative trend in this case.

Theoretical values of the variance may be computed for series I by transforming the above regression formula:

$$\sigma^2_{\delta q} = q(1-q)(1-.506q)/22.18.$$

Comparison of the observed class variances with these values results in considerably better agreement with the observations than is the case using a common estimate (table 12); standard errors were

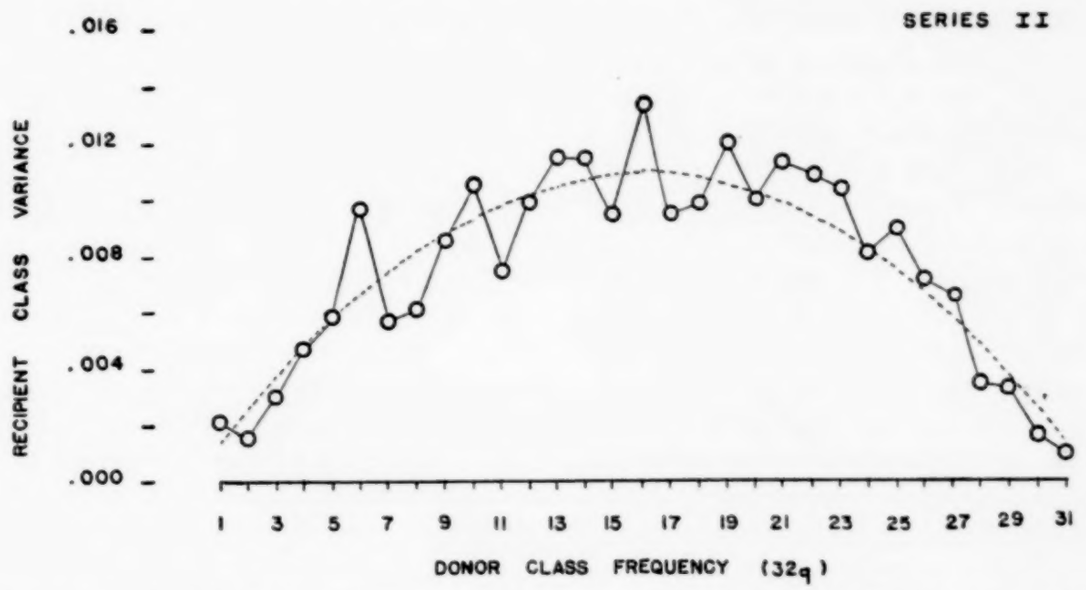

FIG. 3. Comparison of the observed recipient class variances of series II with theoretical values based upon the mean class estimates of $1/2N_e$ (dashed line).

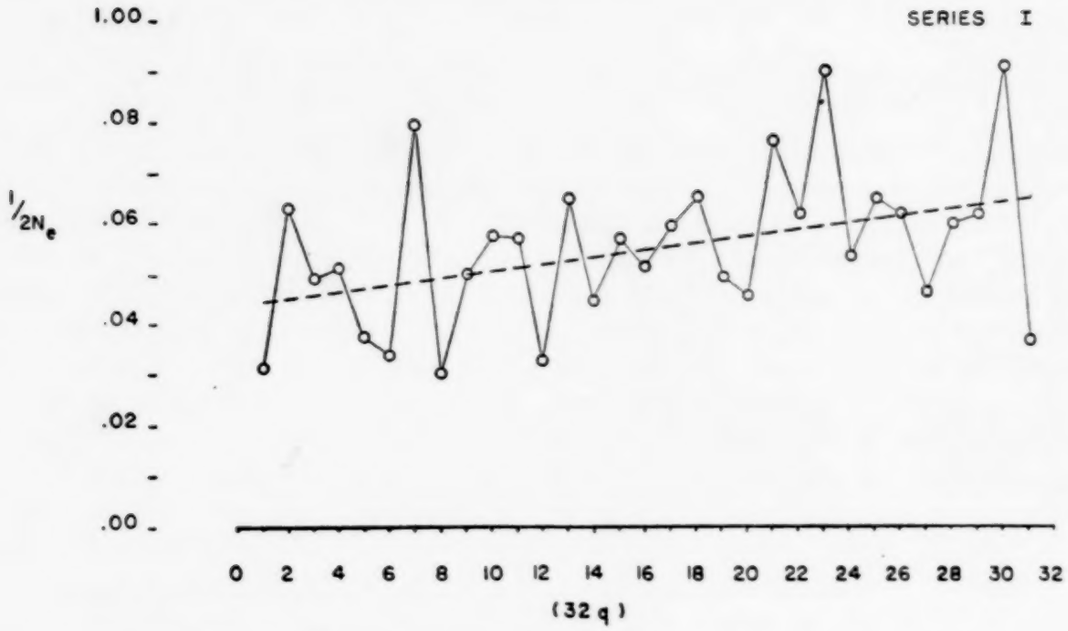

FIG. 4. Observed regression of $1/2N_e$ on q in series I.

computed from the theoretical $2N_e$ values as before. Taking $2N_e$ as a function of q, only three of the points of significance remain, two with probabilities less than .01. The class estimates of series I seem somewhat more variable than would be expected assuming the hypothesis is adequate; however, examination of figure 2 indicates clearly that it is unlikely a refinement of the theory would bring the points of major discrepancy into line. There is apparently some real heterogeneity of an unspecified sort among the frequency classes in this case.

The evident dependence of $2N_e$ on q indicates that the distributions by class are not to be considered purely binomial but rather compounded of binomial elements with variation in productivity associated with genotype.

The Distribution of Gene Frequencies Among Bottles

The histories of the random drift series I and II presented in terms of the distribution of gene frequency by generation for each series appears in tables 14 and 15

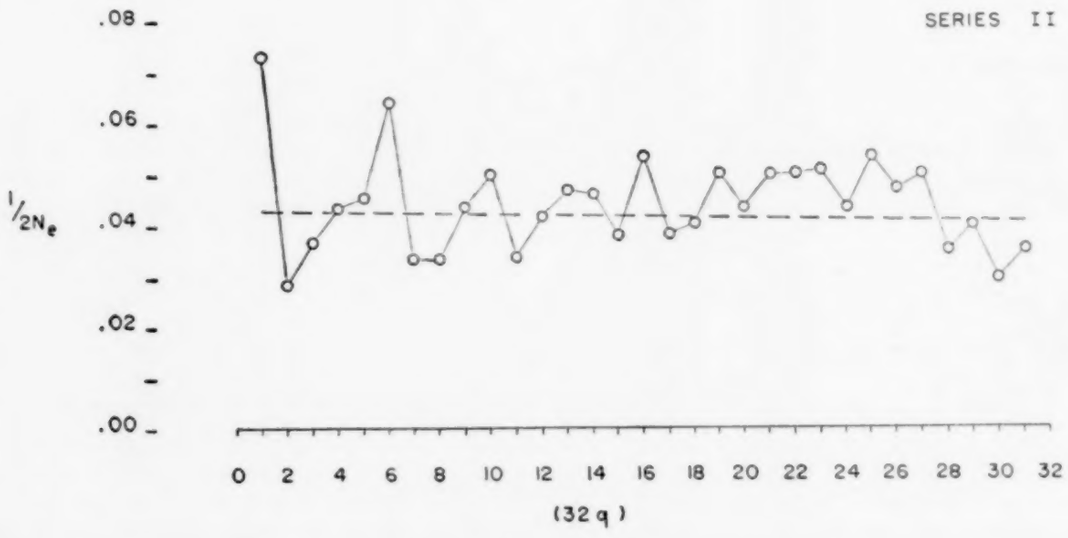

FIG. 5. Observed regression of $1/2N_e$ on q in series II.

TABLE 12. *Series I. Comparison of observed recipient class variances with theoretical values obtained from the calculated linear regression of class estimates of $1/2N_e$ on frequency.* Class value $= 32q_{th}$. All values are presented on a relative scale.

Class value	Obs.	Th.	Diff.	S.E.(th)	t
1	.0010	.0014	−.0004	.0006	.7
2	.0038	.0027	+.0011	.0009	1.2
3	.0043	.0040	+.0003	.0014	.2
4	.0058	.0052	+.0006	.0016	.4
5	.0051	.0064	−.0013	.0021	.6
6	.0053	.0075	−.0022	.0020	1.1
7	.0139	.0086	+.0053	.0018	2.9**
8	.0059	.0095	−.0036	.0022	1.6
9	.0103	.0104	−.0001	.0021	.0
10	.0126	.0112	+.0014	.0021	.7
11	.0132	.0119	+.0013	.0022	.6
12	.0079	.0126	−.0047	.0022	2.1*
13	.0160	.0131	+.0029	.0021	1.4
14	.0112	.0136	−.0024	.0020	1.2
15	.0146	.0139	+.0007	.0020	.4
16	.0131	.0141	+.0010	.0021	.5
17	.0150	.0142	+.0008	.0019	.4
18	.0164	.0143	+.0021	.0021	1.0
19	.0121	.0141	−.0020	.0020	1.0
20	.0109	.0139	−.0030	.0024	1.3
21	.0176	.0135	+.0041	.0026	1.6
22	.0137	.0131	+.0006	.0024	.3
23	.0186	.0124	+.0062	.0023	2.7**
24	.0102	.0117	−.0015	.0021	.7
25	.0113	.0108	−.0005	.0026	.2
26	.0097	.0097	−.0000	.0021	.0
27	.0063	.0085	−.0022	.0020	1.1
28	.0061	.0071	−.0010	.0016	.6
29	.0054	.0056	−.0002	.0014	.1
30	.0054	.0039	+.0015	.0010	1.5
31	.0012	.0020	−.0008	.0008	1.0

and figures 6 and 7. The class values, ranging from 0–32, are gene frequencies on the absolute scale determined by the controlled sample size of 16 flies per bottle (32 genes).

The distributions of series I and II are evidently very similar. Certain differences are, however, apparent. The spread in the range of observed frequencies following the initiation of each series is somewhat more rapid in series I. The diagrams in the early generations of series II are positively skewed reflecting the consistent positive value of $\overline{\Delta q}$ over the first 4 generations as already noted. The stable form of the frequency distribution, which is theoretically approximately uniform in the absence of systematic pressures and assuming a constant value of $1/2N_e$ for all classes (Fisher 1930, Wright 1931), is sensibly approached in generation 11 in series I while in series II certainly not before generation 17.

After stability of form has been reached, the fixation rate is theoretically uniform $(1/2N_e)$ (Wright, 1931). A chi-square applied to the observed total number of bottles fixed over the last 9 generations of series I, basing the calculated values on the ratio of the number of bottles observed to reach fixation to the total number exposed to fixation over this period (.0676) gives a value of $\chi^2 = 6.08$, $n = 8, p = 0.50 - 0.70$. The stable form in series II cannot have been represented for a number of generations sufficient to warrant consideration of the observed fixation rate.

In the case of series I there is a strong suggestion of a negative regression of frequency on class value. The grouped frequency distribution over the last 9 generations (fig. 8) bears this out. A trend line fitted to these data, omitting the newly fixed classes and ignoring the obvious non-linearity, has a coefficient, $b = -.024$, which is significant with a probability of about 0.02, $t = 2.38$, $n = 29$. Despite the non-random distribution of signs about the fitted line, this method gives a rough estimate of the significance of this tendency. The effect is clearly related to the observed dependence of $1/2N_e$ on q in this case.

The grouped frequency distribution over the last three generations of series II appears in figure 9. There is here no suggestion of slope. The stable distribution may be taken to be uniform.

Considering the probable significance of the positive value of $\overline{\Delta q}$ obtained from the whole body of the data of series II and the asymmetry of the distributions in the earlier generations it is at first sight surprising that there should have been no definite slope to the terminal distributions. However, the marked

TABLE 13. *Series I. Distributions of numbers of* bw^{75} *genes among the cultures of each generation and grouped for generations (11–19) and (1–19).*

Generation	Total fixed *bw*	Number of *bw*^{75} genes																																	Total fixed *bw*^{75}	Total newly fixed	Total frequency	Fixation rate
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32				
0																		107																				
1									1	0	0	0	2	5	8	10	13	22	9	14	14	7	1	1														
2				1	0	0	0	2	3	1	1	4	8	7	7	2	15	9	8	13	7	5	3	1	2	4	1	0	2	0	1							
3					2	0	1	1	2	3	5	3	4	9	8	8	9	8	6	7	4	7	2	5	2	2	2	2	3	0	0	1	1					
4					2	0	2	1	5	4	6	8	6	6	2	5	8	9	3	3	7	4	3	3	6	4	2	2	2	0	1	2	0	1	1	1	107	0.0093
5				1	1	2	3	5	3	6	5	1	6	4	5	4	4	5	10	7	5	3	4	3	2	1	4	5	3	1	0	2	0	1	2	1	106	0.0094
6	1	1	1	2	1	2	2	3	6	2	10	7	2	4	4	7	4	9	4	2	1	4	6	7	0	4	0	3	3	2	0	1	0	1	3	2	105	0.0190
7	3	2	2	1	0	0	1	5	5	7	5	2	4	3	5	12	5	5	5	4	5	1	1	3	1	2	5	1	2	7	1	1	0	0	3	2	103	0.0194
8	5	2	0	1	1	2	3	4	4	2	10	3	4	3	4	5	8	6	6	3	2	2	5	3	1	3	1	0	0	3	2	3	3	2	5	4	101	0.0396
9	5	0	1	2	5	3	2	1	3	5	3	7	7	5	3	4	2	5	4	2	1	4	4	3	3	1	3	3	2	3	1	1	3	1	6	1	97	0.0103
10	7	2	2	4	3	5	0	3	1	4	2	3	2	4	5	3	4	3	6	7	4	5	2	1	5	1	2	2	1	2	2	2	2	2	8	4	96	0.0417
11	11	4	1	1	3	6	3	5	2	1	2	1	3	2	2	3	5	4	2	3	5	3	4	5	5	2	2	0	2	2	2	2	3	2	10	6	92	0.0652
12	12	1	1	0	4	6	2	2	4	2	2	1	3	0	6	3	5	1	4	4	2	4	5	3	2	4	3	0	1	2	1	0	1	7	17	8	86	0.0930
13	12	0	4	3	3	4	0	3	3	2	4	1	3	3	0	2	1	7	5	4	3	4	2	1	2	1	2	2	2	2	1	2	1	1	18	1	78	0.0128
14	15	3	6	1	5	2	0	3	1	2	2	4	1	3	3	3	1	6	3	5	2	2	0	0	1	2	2	3	2	1	2	3	0	3	21	6	77	0.0779
15	18	3	4	4	2	3	3	0	0	1	1	5	2	5	3	1	4	4	1	1	0	2	2	0	1	2	1	6	0	2	2	1	3	2	23	5	71	0.0704
16	23	5	0	2	4	1	3	2	1	1	2	1	2	2	7	1	2	2	3	3	2	2	0	1	0	2	0	2	0	4	3	1	3	2	25	7	66	0.1061
17	26	3	0	1	4	1	2	4	1	4	1	0	0	3	3	3	2	3	1	2	2	2	0	2	2	1	2	1	2	1	2	2	1	1	26	4	59	0.0678
18	27	1	3	1	3	1	4	2	0	0	3	1	1	1	4	2	3	1	3	2	0	2	2	3	0	3	1	3	0	0	1	1	1	2	28	3	55	0.0545
19	30	3	2	0	3	1	2	0	5	2	3	1	1	1	5	2	0	0	2	0	2	2	3	2	1	2	2	1	0	0	0	0	4	0	28	3	52	0.0577
1–19		30	27	25	46	39	33	46	50	49	67	53	61	70	84	80	95	109	85	86	68	65	49	47	36	41	35	36	27	32	22	25	26	28			1672	
11–19		23	21	13	31	25	19	21	17	15	20	15	16	20	33	20	23	28	24	24	18	23	18	17	14	19	15	18	9	14	14	12	17	20			636	

TABLE 14. *Series II. Distributions of numbers of* bw^{75} *genes among the cultures of each generation and grouped for generations (17–19) and (1–19).*

Generation	Total fixed *bw*	Number of *bw*^{75} genes																																		Total fixed *bw*^{75}	Total newly fixed	Total frequency	Fixation rate
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32				
0																	105																					
1											2	1	1	6	6	16	10	14	12	13	9	7	5	1	0	2											105	
2											5	2	6	4	9	8	11	6	5	16	10	4	3	6	3	3	1	0	2	1							105	
3							1	0	1	2	0	2	2	6	9	7	11	9	8	4	10	6	5	5	4	2	2	2	5	1	1						105	
4						1	0	0	1	3	2	3	0	5	4	9	5	6	8	8	8	7	5	4	7	5	5	2	2	0	1	3	1				105	
5				1	0	2	0	1	3	1	0	4	6	2	8	6	6	3	5	6	6	6	4	6	5	2	2	5	1	6	3	2	1	1	1	1	104*	0.0096
6				2	0	0	1	2	1	2	2	2	4	7	6	4	4	7	7	5	7	1	2	5	4	5	2	2	1	6	6	4	1	1	2	1	103	0.0097
7			1	1	0	3	0	0	4	1	2	7	3	1	5	7	7	10	7	1	3	3	2	2	1	4	5	3	3	6	2	2	4	2	4	2	102	0.0196
8	1	1	1	2	0	1	1	3	2	4	2	7	6	0	2	4	4	5	5	6	4	4	2	4	3	6	1	5	2	1	2	4	2	4	8	5	100	0.0500
9	2	1	1	0	2	1	2	1	3	5	3	3	2	4	3	3	3	5	8	7	0	3	5	2	8	3	2	5	1	1	1	3	3	1	9	2	95	0.0211
10	3	1	1	1	1	0	2	1	8	0	3	3	2	3	1	4	7	2	5	4	7	5	3	4	5	4	0	3	2	1	4	4	1	1	10	2	93	0.0215
11	3	0	1	3	2	1	1	2	1	4	3	1	2	2	3	3	6	5	6	3	7	3	3	4	3	1	2	2	6	1	2	5	2	1	11	1	91	0.0110
12	3	0	2	2	3	1	2	2	4	3	3	1	3	1	2	5	3	5	3	2	5	4	2	4	4	3	1	1	5	3	4	4	1	2	13	2	90	0.0222
13	5	2	3	2	1	2	1	5	0	1	4	0	4	4	6	4	2	1	3	3	3	6	1	3	2	5	3	2	1	3	5	1	3	2	15	4	88	0.0455
14	5	0	2	3	1	1	4	1	4	3	2	2	4	2	1	2	4	4	5	3	6	1	2	5	0	6	0	2	1	4	2	1	1	5	20	5	84	0.0595
15	7	2	1	2	4	4	0	0	5	2	2	4	2	6	3	1	6	3	2	4	1	2	2	0	1	3	3	3	2	1	2	3	2	1	21	3	79	0.0380
16	7	0	3	2	3	0	3	3	2	1	4	6	1	4	6	2	0	2	6	1	1	2	3	2	1	1	0	3	2	4	3	2	1	2	23	2	76	0.0263
17	11	4	2	2	1	2	1	4	2	6	2	2	3	3	1	3	2	1	5	0	3	2	3	0	3	0	3	2	2	3	1	1	4	1	24	5	74	0.0676
18	13	2	0	1	2	3	1	4	3	0	5	3	4	1	1	2	3	1	2	2	3	0	0	2	2	7	1	3	0	2	0	3	0	6	30	8	69	0.1159
19	13	0	1	3	2	2	2	3	1	1	3	4	2	1	1	3	3	3	3	1	1	2	1	0	1	2	5	1	1	1	5	2	0	0	30	0	61	0.0000
1–19		13	19	27	22	24	22	32	45	39	49	57	57	62	77	93	97	92	105	89	94	68	53	59	57	64	38	46	39	45	44	44	27	30			1729	
17–19		6	3	6	5	7	4	11	6	7	10	9	9	5	3	8	8	5	10	3	7	4	4	2	6	9	9	6	3	6	6	6	4	7			204	

* Accidental loss.

asymmetry of generations 1 to 4, reflecting the period of initial increase in the frequency of *bw*^{75}, is largely dissipated by the 15th generation due wholly to a drop in gene frequency among unfixed classes brought about by the asymmetry in fixation, as has already been pointed out.

The theoretical distribution in the case of series I in which a constant value of $1/2N_e$ cannot be assumed may be obtained from the Fokker-Plank equation (Wright, personal communication):

$$\frac{1}{2}\frac{\partial^2}{\partial q^2}\left[\sigma^2_{\delta q}\phi(q, t)\right] - \frac{\partial}{\partial q}\left(\Delta q \phi(q, t)\right) = \frac{\partial \phi(q, t)}{\partial t}$$

Considering the distribution only after the stable form and, accordingly, a con-

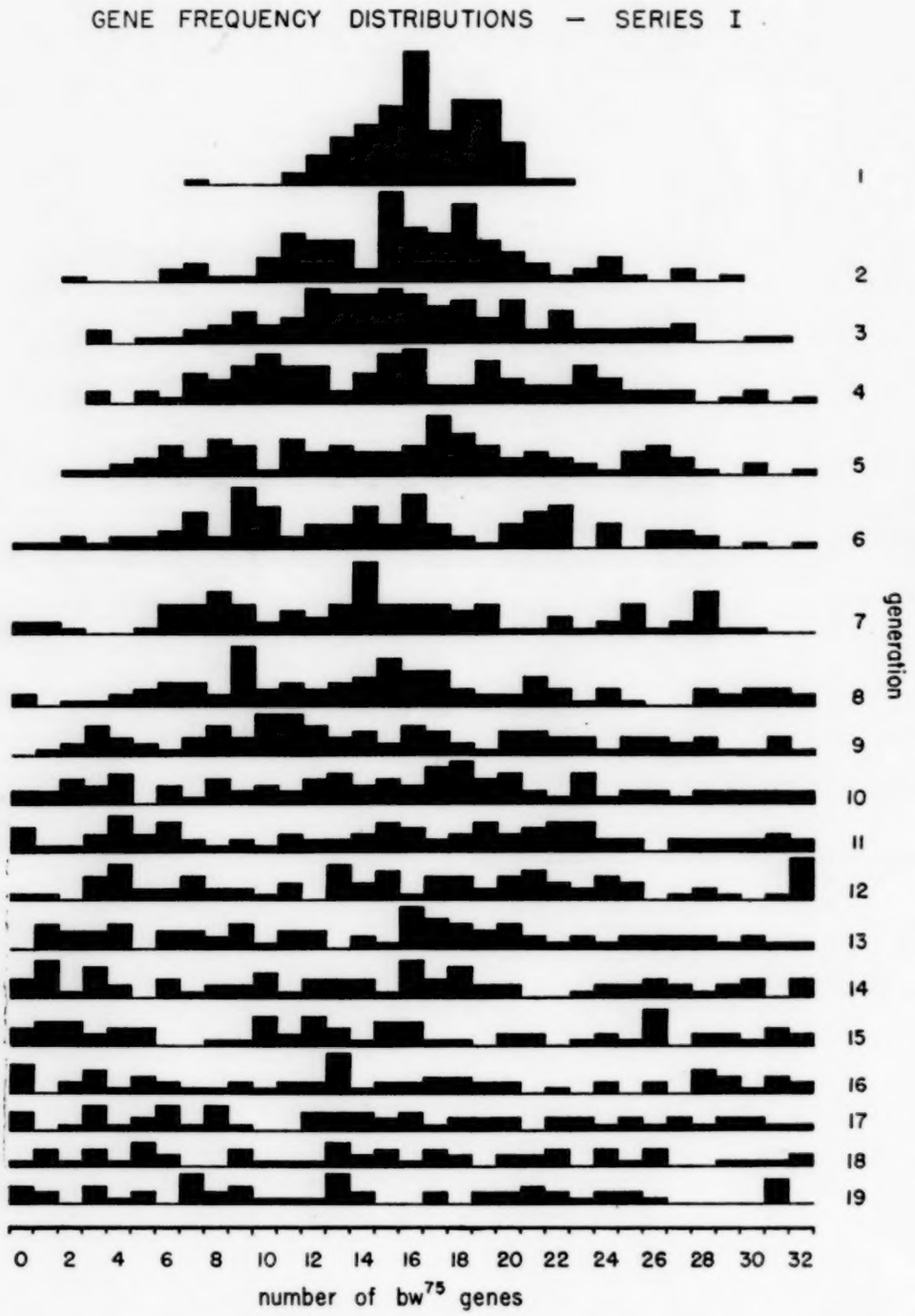

FIG. 6. Gene frequency distributions by generation in series I. Graphical presentation of the data of table 13.

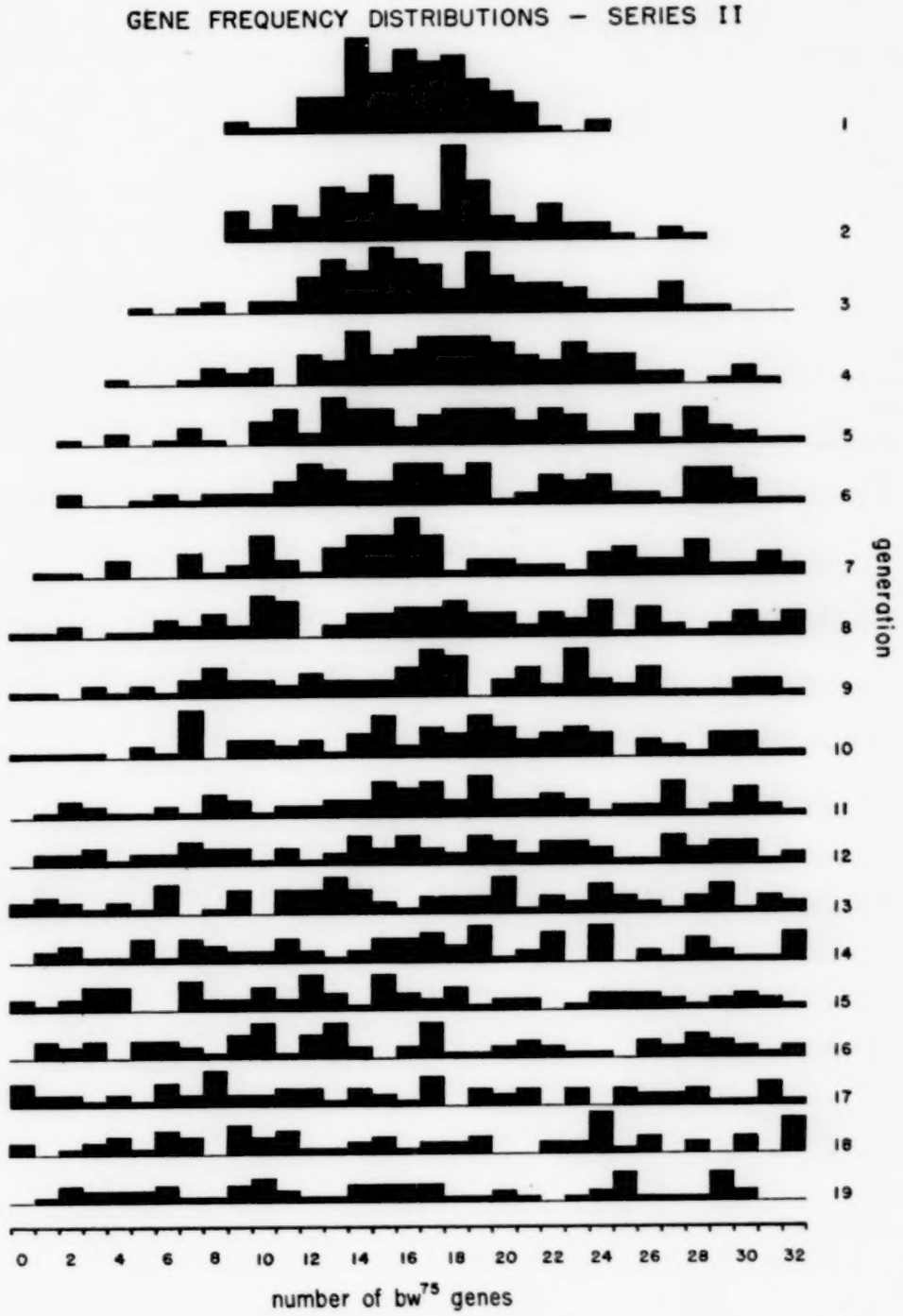

FIG. 7. Gene frequency distributions by generation in series II. Graphical presentation of the data of table 14.

stant rate of decay has been reached, this reduces to the ordinary differential equation:

$$\frac{1}{2}\frac{d^2}{dq^2}\left(\sigma^2_{\delta q}\phi(q)\right) - \frac{d}{dq}\left(\Delta q\,\phi(q)\right) + k\phi(q) = 0$$

(Wright, 1945). In the present case in which systematic forces may be neglected, the second term drops out ($\Delta q = 0$) and the equation to be solved for $\phi(q)$, is:

$$\frac{1}{2}\frac{d^2}{dq^2}\left(\sigma^2_{\delta q}\phi(q)\right) + k\phi(q) = 0$$

Let

$$\phi(q) = C(1 + C_1q + C_2q^2 + C_3q^3 \cdots C_nq^n)$$

and

$$\sigma^2_{\delta q} = q(1-q)(1+bq)/2N_0,$$

TABLE 15. *Computed frequency distribution for series I.*

32q	$\phi(q)/C$	%	32q	$\phi(q)/C$	%	32q	$\phi(q)/C$	%
0	.7214	3.01	11	.7885	3.28	22	.6406	2.67
1	.9772	4.07	12	.7729	3.22	23	.6293	2.62
2	.9552	3.98	13	.7578	3.16	24	.6183	2.58
3	.9340	3.89	14	.7432	3.10	25	.6077	2.53
4	.9136	3.81	15	.7290	3.04	26	.5975	2.49
5	.8939	3.72	16	.7152	2.98	27	.5875	2.45
6	.8749	3.64	17	.7018	2.92	28	.5777	2.41
7	.8565	3.57	18	.6889	2.87	29	.5682	2.37
8	.8387	3.49	19	.6763	2.82	30	.5590	2.33
9	.8214	3.42	20	.6640	2.77	31	.5499	2.29
10	.8047	3.35	21	.6521	2.72	32	.5877	2.45

where N_0 = the effective population number at q = 0. Then,

$$\frac{d^2}{dq^2}[q(1-q)(1+bq) \times (1+C_1q+C_2q^2+C_3q^3\cdots)] = -4N_0k(1+C_1q+C_2q^2+C_3q^3\cdots)$$

Arranging by powers of q and differentiating,

$$2[C_1-(1-b)]+6[C_2-C_1(1-b)-b]q +12[C_3-C_2(1-b)-C_1b]q^2\cdots +n(n+1)[C_n-C_{n-1}(1-b)-C_{n-2}b]q^{n-1} = -4N_0k(1+C_1q+C_2q^2 +C_3q^3\cdots C_{n-1}q^{n-1})$$

Equating the coefficients of like powers of q,

$$C_1 = (1-b) - 2N_0k$$
$$C_2 = b + (1-b)C_1 - [2N_0kC_1/3]$$
$$C_3 = bC_1 + (1-b)C_2 - [2N_0kC_2/6]$$
$$C_n = bC_{n-2} + (1-b)C_{n-1} - [4N_0kC_{n-1}/n(n+1)]$$

The system of coefficients must be evaluated by finding by repeated trial the value of $2N_0k$ that will make the series convergent, which requires that the coefficients be kept alternating in sign. It has been found (Wright, personal communication) that, with b = .506, $2N_0k = 1.2375$ is correct to 4 decimals and permits the accurate computation of

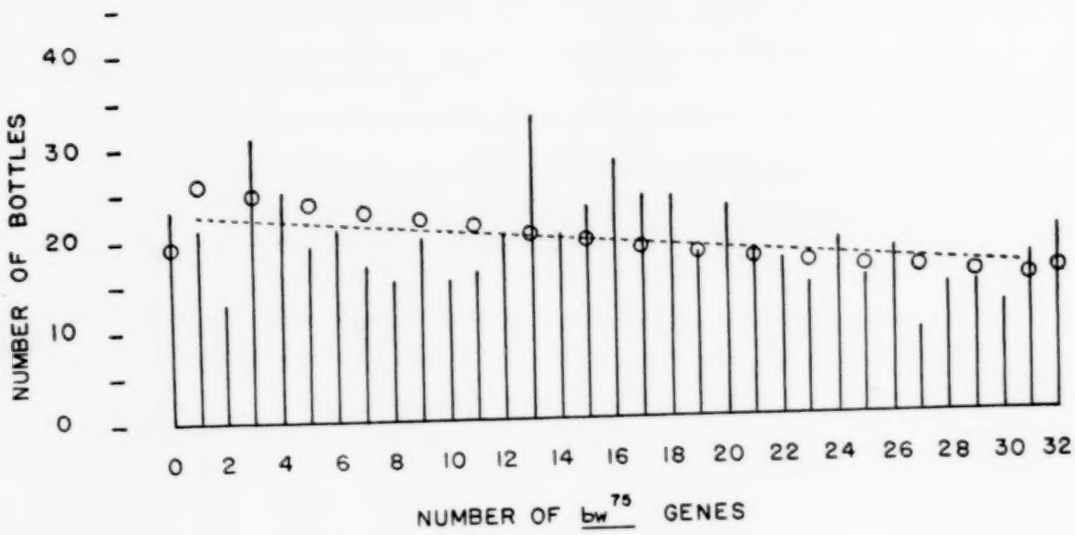

FIG. 8. Cumulative frequency distribution over the last 9 generations of series I. The dashed line represents the equation of linear trend. The open circles indicate theoretical values obtained from the frequency function, $\phi(q)$, based on the regression of $1/2N_e$ on q.

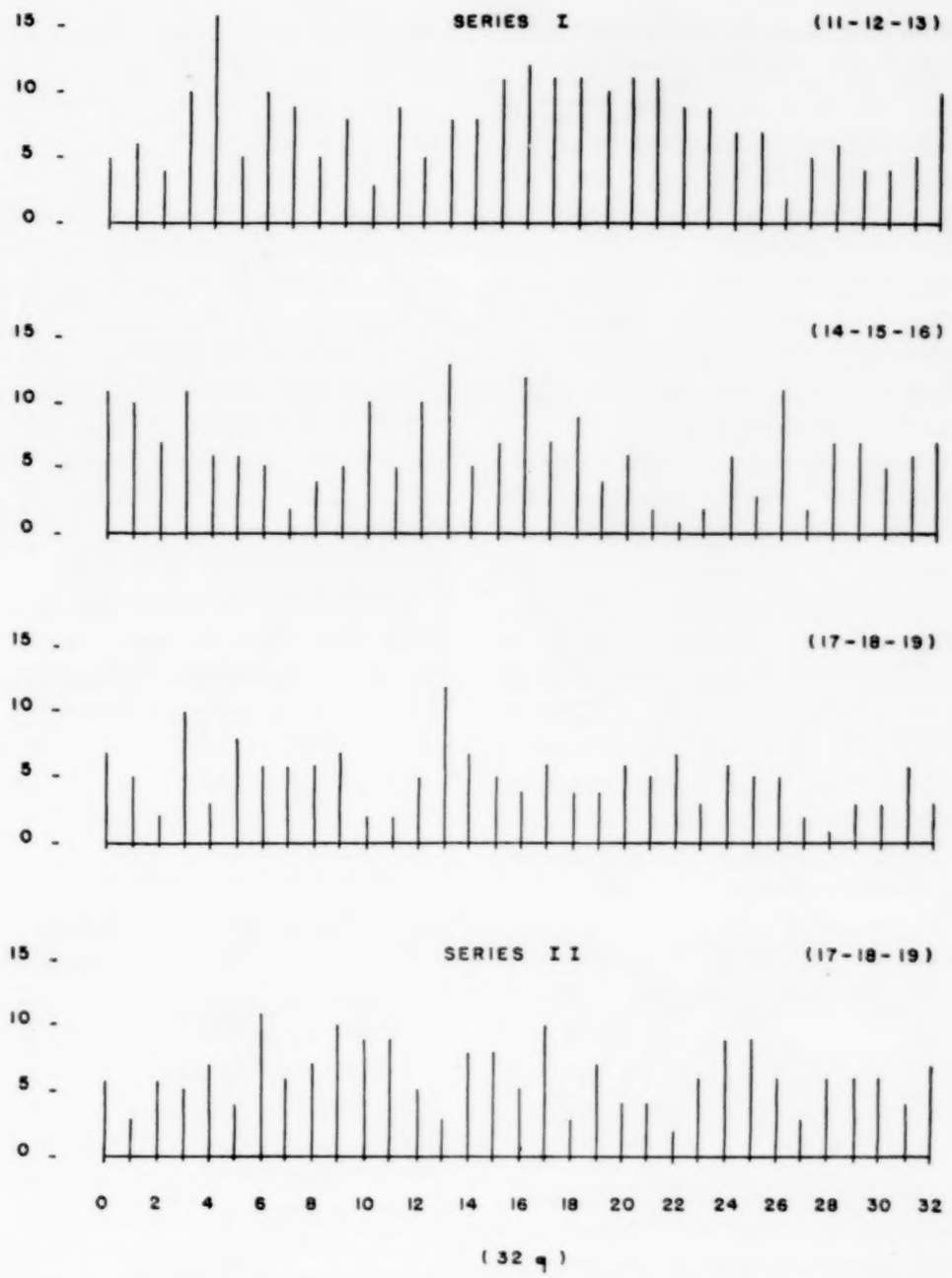

FIG. 9. Grouped frequency distribution from the data of series I over the last 3 consecutive groups of 3 generations together with the distribution over the last 3 generations of series II.

the coefficients to four places up to C_{15}. The equation for the distribution of gene frequencies may be written with sufficient accuracy:

$$\begin{aligned}\phi(q) = C(1 &- .7435q + .4454q^2 - .2481q^3 \\ &+ .1335q^4 - .0706q^5 - .0369q^6 \\ &- .0191q^7 + .0099q^8 - .0051q^9 \\ &+ .0026q^{10} - .0013q^{11} + .0007q^{12} \\ &- .0004q^{13} + .0002q^{14} - .0001q^{15})\end{aligned}$$

The terminal frequencies may be found to be $\phi(0) = C$ and $\phi(1) = .5410C$ respectively.

The total fixation rate at the two extremes is equal to the rate of decay of the unfixed classes. For the present value of $2N_0k$, $k = .0558$, taking $2N_0 = 22.18$ from the calculated regression of $1/2N_e$ on q. This value is .996 of $\overline{(1/2N_e)}$ and thus only slightly less than the expected value in the case of uniform class

frequencies computed on the basis of the average class value, $\overline{[\sigma^2_{\delta q}/q(1-q)]}$.

This method of determining the frequency distribution is in error near the extremes. Fisher (1930) has given the correction factors in the case of uniform uncorrected frequencies. Similar corrections would be required in the present case for an exact result but the uncorrected ordinates at multiples of the theoretical class range (1/32) may be taken as a sufficient approximation.

It has been shown (Wright, 1931; Wright and Kerr, 1954) that good approximations of the rate of fixation of each allele may be taken to be half the proportional frequency in the uncorrected subterminal classes. In the present case in which $2N_e$ varies, the *effective* class ranges must be taken as $1/2N_0 = 1/22.18$ adjacent to $q=0$, and as $1/2N_1 = 1/14.73$ adjacent to $q = 1$. The corresponding ordinates may be taken as $\phi(0) = C$ and $\phi(1) = .5410C$ from the formula as given. The approximate theoretical rates of fixation are then:

$$k_0 = 1/2\, f_{0(e)} = 1/2[C/22.18] = .7214C/32$$

$$k_1 = 1/2\, f_{1(e)} = 1/2[.5410C/14.73] = .5877C/32$$

The corresponding theoretical relative frequencies for the unfixed classes, on a scale with class ranges of 1/32, may be estimated as $\phi(q)/32$. Table 15 gives the relative frequencies in the form $\phi(q)/C$ and the same values on a percentage scale. It should be noted that the estimated total proportion fixed per generation (.0546) is in fair agreement with the rate (.0558) obtained in solving the general equation (which applies strictly when $2N \to \infty$).

It remains to consider the observed distributions in the light of the theoretical in both cases. Figure 8 presents the calculated distribution for series I in comparison with the observed frequencies summed over the last 9 generations. The theoretical distribution has been adjusted to a total frequency of 636.

There is a rather close agreement between this curve and the simple linear trend line although the former is based solely on the observed recipient class variances. The calculated curve results in no improvement with respect to the distribution of the signs associated with the observed deviations. There is an obvious clumping of high class frequency in the middle range and a dearth of frequency in the lower frequency classes. A chi-square test (table 16) for goodness of fit gives a value of 42.28 with 31 degrees of freedom, $p = 0.10$. The signs of the differences bring out again the discrepancies already mentioned. Grouping the 33 classes into 6 alternating groups of 5 and 6 frequency classes, $\chi^2 = 14.15$, with 5 degrees of freedom, $p = 0.01 - 0.02$; using 3 groups of 11 classes each, $\chi^2 = 11.57$, $n = 2$, $p = 0.001 - 0.01$. The decrease in the probability as the groups are made larger is entirely due to the non-random distribution of signs.

There are several suggestions that can be made regarding the rather poor accord with expectation in this case. It has been shown theoretically (Kimura, 1955) that in cases such as are considered here it takes about $2N_e$ generations to reach substantial equilibrium with respect to form. The average value of $2N_e$ in series I was about 18 while at the upper and lower extremes the values were about 15 and 22 respectively. From this it appears that the upper half of the distribution should not be found in good accord with expectation much before the 15th generation and the lower half not before the end of the experiment.

Comparison of successively smaller terminal groupings shows that the preponderance of negative deviations in the upper half of the range of frequency classes is alleviated somewhat in the grouping of generations 15–19, and progressively improves in the grouping of generations 16–19 and 17–19. However, the distribution as a whole undergoes

TABLE 16. *Chi-square comparing the observed class frequencies over generations 1–19 in series I with values obtained from the frequency distribution, $\phi(q)$, calculated on the bases of the observed regression of $1/2N_e$ on q.* Tests are presented for the ungrouped distributions, for groupings into alternating groups of 5 and 6 classes, and for 3 groups of 11 classes each.

32q	o	c	o−c	$\frac{(o-c)^2}{c}$	o	c	o−c	$\frac{(o-c)^2}{c}$	o	c	(o−c)	$\frac{(o-c)^2}{c}$
0	23	19.1	+ 3.9	.80								
1	21	25.9	− 4.9	.93								
2	13	25.3	−12.3	5.98								
3	31	24.7	+ 6.3	1.61								
4	25	24.2	+ 0.8	.03	113	119.2	− 6.2	.32				
5	19	23.7	− 4.7	.93								
6	21	23.2	− 2.2	.21								
7	17	22.7	− 5.7	1.43								
8	15	22.2	− 7.2	2.34								
9	20	21.8	− 1.8	.15								
10	15	21.3	− 6.3	1.86	107	134.9	−27.9	5.77	220	254.1	−34.1	4.57
11	16	20.9	− 4.9	1.15								
12	20	20.5	− 0.5	.01								
13	33	20.1	+12.9	8.28								
14	20	19.7	+ 0.3	.00								
15	23	19.3	+ 3.7	.71	112	100.5	+11.5	1.32				
16	28	18.9	+ 9.1	4.38								
17	24	18.6	+ 5.4	1.57								
18	24	18.2	+ 5.8	1.85								
19	18	17.9	+ 0.1	.00								
20	23	17.6	+ 5.4	1.66								
21	18	17.3	+ 0.7	.03	135	108.5	+26.5	6.47	247	209.0	+38.0	6.91
22	17	17.0	0.0	.00								
23	14	16.7	− 2.7	.44								
24	19	16.4	+ 2.6	.41								
25	15	16.1	− 1.1	.08								
26	18	15.8	+ 2.2	.31	83	82.0	+ 1.0	.01				
27	9	15.6	− 6.6	2.79								
28	14	15.3	− 1.3	.11								
29	14	15.0	− 1.0	.07								
30	12	14.8	− 2.8	.53								
31	17	14.6	+ 2.4	.39								
32	20	15.6	+ 4.4	1.24	86	90.9	− 4.9	.26	169	172.9	− 3.9	.09
	636	636.0	0.0	42.28	636	636.0	0.0	14.15	636	636.0	0.0	11.57

some distortion during this period. Figure 9 presents a breakdown of the frequency distributions of generations 11–19 into 3 successive groups of 3. It is evident that the "rarefaction" in the gene frequency range 4–13 of (11–12–13) is enhanced in the next group (14–15–16) wherein another "rarefaction" in the region of frequency 19–26 appears. The terminal grouping (17–18–19) shows a restoration of these secondary imbalances. Such fluctuations have doubtless, in the present case, delayed the attainment of satisfactory agreement with the theoretical distribution. Further comment on this point will be made in the discussion.

Grouping the last 3 generations of series II a χ^2 for goodness of fit on the hypothesis of uniformity gives a value

of 28.29 with 32 degrees of freedom $p = 0.50$. Though the estimated value of $2N_e$ in this case is about 23, there is only a suggestion (fig. 9) of higher frequencies in the middle range and the deviations are well distributed about the mean value with respect to sign.

Total Variance and the Proportion of Heterozygosis

In a subdivided diploid population, with random breeding within the subunits but complete isolation between them, a decrease in the proportion of heterozygosis and an increase in the total variance of gene frequency among subpopulations are expected in each generation (Wright, 1931). Both can be calculated as functions of $2N_e$. It is of interest to compare the rates of change in each of these factors with theoretical rates based on the values of $2N_e$ obtained from consideration of the variances of recipient class frequencies in the last section. In the following computations values of $2N_e$ of 18 and 23 were taken as sufficiently accurate estimates for series I and II respectively. This should be adequate even in the case of series I despite the fact of regression since $\overline{2N_e}$ is close to the computed value of $2N_e$ for class 16. Selection is assumed to have been absent throughout.

The expected proportion (P) of heterozygotes relative to that in a very large undivided population under random mating and at the same gene frequency may be calculated by generation using the following formula:

$$P = P' - 1/2N_e(2P' - P''),$$

where P' and P'' are the proportions in the first and second preceding generations respectively (Wright, 1931). The percentage of heterozygotes expected in any given generation (n) is given by $P_n 2q_0(1 - q_0)$, where q_0 is the initial gene frequency. $P = (1 - F)$, where F, the inbreeding coefficient, measures the correlation between uniting gametes. Since all cultures were started with heterozygous flies, the gametes uniting to form the foundation population show perfect negative correlation, $P_0 = 2$. $P_1 = 1$, because only segregation is involved. Since all populations in generation 1 are identical, there will be no overall correlation between uniting gametes if mating is random within cultures, thus P_2 will also be 1. Subsequently the value of P

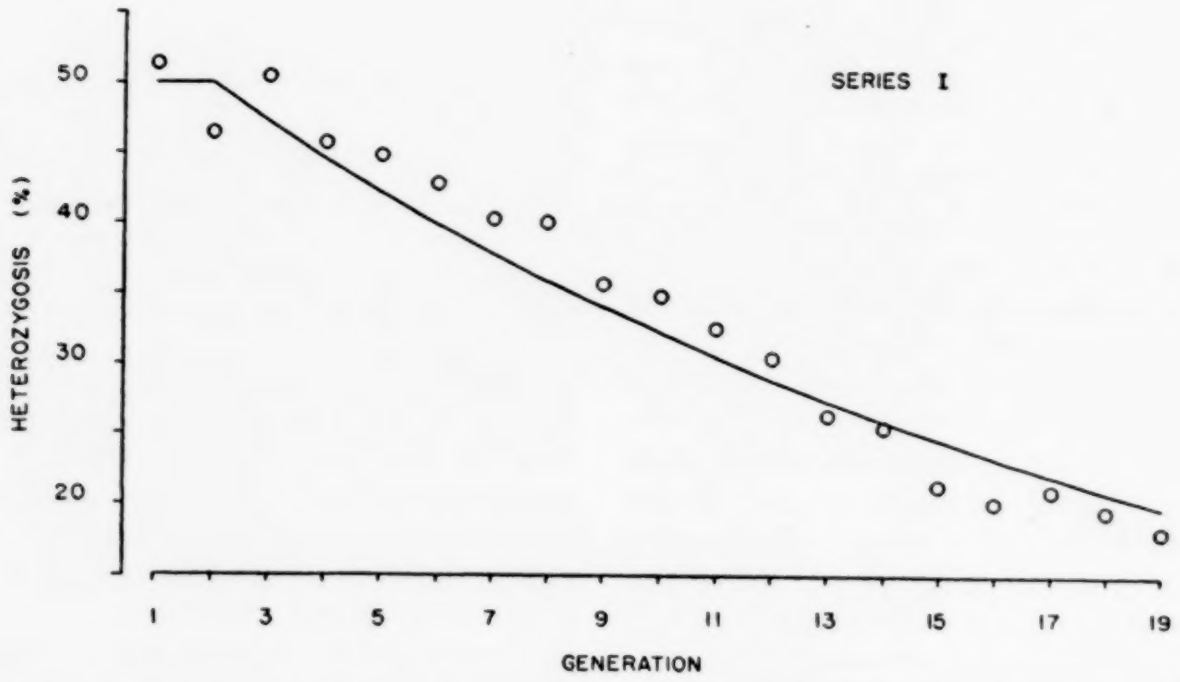

FIG. 10. Series I. Comparison of observed heterozygosis in percent (open circles) with expected values based on a common estimate of $2N_e = 18$ (smooth line).

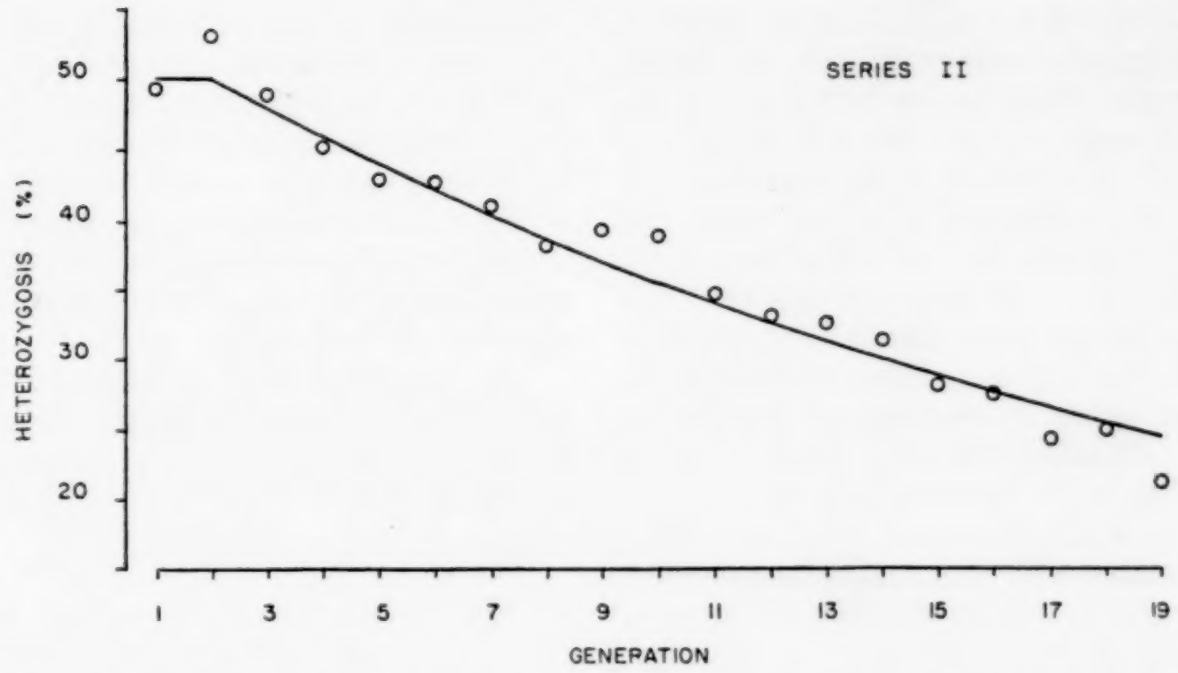

FIG. 11. Series II. Comparison of observed heterozygosis in percent (open curves) with expected values based on a common estimate of $2N_e = 23$ (smooth line).

decreases due to accidents in the sampling of 2N gametes.

A comparison of the observed with the calculated values of the percentage of heterozygosis appears in figures 10 and 11 and table 17. It is evident from examination of the figure that the agreement with expectation is somewhat better

TABLE 17. *Proportion of heterozygosis and total gene frequency by generation.* Theoretical values were calculated assuming $2N_e$ values of 18 and 23 for series I and II respectively. The expected proportion of heterozygotes in % was computed as $P_n\ 2q_0(1 - q_0)$, where $q_0 = 0.50$ and is compared by generation with the observed values. The computed total variances are compared in each generation with the observed variance both including (A) and excluding (B) previously fixed classes.

	Heterozygosis						Total variance					
	Series I			Series II			Series I			Series II		
Gen.	P	Exp. (%)	Obs. (%)	P	Exp. (%)	Obs. (%)	σ^2_{th}	σ^2_A	σ^2_B	σ^2_{th}	σ^2_A	σ^2_B
0	2.00	100.0	100.0	2.00	100.0	100.0	.000	.000	.000	.000	.000	.000
1	1.00	50.0	51.4	1.00	50.0	49.2	.014	.006	.006	.011	.009	.009
2	1.00	50.0	46.4	1.00	50.0	52.9	.027	.026	.026	.021	.018	.018
3	.94	47.0	50.4	.96	48.0	48.5	.039	.031	.031	.031	.024	.024
4	.90	45.0	45.6	.92	46.0	45.1	.051	.042	.042	.041	.030	.030
5	.85	42.5	44.8	.88	44.0	42.7	.062	.050	.048	.050	.045	.045
6	.80	40.0	42.8	.84	42.0	42.6	.073	.055	.051	.059	.052	.050
7	.76	38.0	40.3	.81	40.5	40.8	.082	.062	.054	.067	.060	.058
8	.72	36.0	40.2	.77	38.5	38.0	.092	.072	.062	.075	.070	.065
9	.68	34.0	35.8	.74	37.0	39.1	.101	.083	.066	.083	.072	.059
10	.65	32.5	34.8	.71	35.5	38.6	.109	.090	.071	.090	.076	.059
11	.61	30.5	32.5	.68	34.0	34.6	.117	.105	.081	.097	.084	.064
12	.58	29.0	30.5	.65	32.5	33.1	.124	.112	.077	.103	.094	.074
13	.55	27.5	26.3	.63	31.5	32.5	.131	.123	.073	.110	.101	.079
14	.52	26.0	25.5	.60	30.0	31.3	.138	.136	.089	.116	.105	.075
15	.49	24.5	21.6	.57	28.5	28.0	.144	.140	.096	.122	.117	.078
16	.47	23.5	20.2	.55	27.5	27.5	.150	.155	.095	.127	.122	.079
17	.44	22.0	21.0	.53	26.5	24.3	.155	.160	.084	.133	.133	.088
18	.42	21.0	19.7	.51	25.5	24.9	.161	.165	.082	.138	.139	.089
19	.40	20.0	18.3	.48	24.0	21.0	.166	.170	.081	.143	.144	.077

in the case of series II than for series I. Since the process is stochastic the appropriate comparisons are between the apparent slopes rather than between the observed differences in each generation. There is no inherent tendency to correct for shifts due to sampling accidents. The slopes of the observed and theoretical curves are quite comparable in series II. Following an exceptionally high observed value in the second generation there is a suggestion of a greater rate of decrease than expected for about four generations. However, the rate over generations 4 to 14 is in good accord with expectation. After this time it appears to increase slightly again.

In series I the agreement is perhaps better than appears at first sight. The observed rate after the rather erratic fluctuations of the first three generations is in good accord with expectation up to generation 12. The curves, although not coincident, are roughly parallel during this period. After this the rate appears to increase somewhat, reflecting sharp decreases in observed heterozygosis between generations 12 & 13 and 14 & 15.

It should be pointed out again that the theoretical curves are based upon the assumption of a uniform value of $2N_e$ throughout the course of the experiment. Some heterogeneity in the average value of this parameter among generations in finite populations would be expected and would result in the sort of loose "fit" apparent in both cases.

The expected total variance of gene frequency among populations, including those fixed, is given for any generation by the formula:

$$\sigma_q^2 = q_0(1-q_0)[1-(1-1/2N_e)^n]$$

(Wright, 1942), where q_0 is the initial frequency and n the number of generations. The total variance of q among bottles including both fixed and unfixed cultures reaches a maximum when all bottles are fixed for one or the other of the two alternatives. The theoretical variance for a given generation excluding the previously fixed cultures rises to a maximum value equal to the variance of the stable form of the frequency distribution. The hypothesis that the stable form in these cases is approximated by

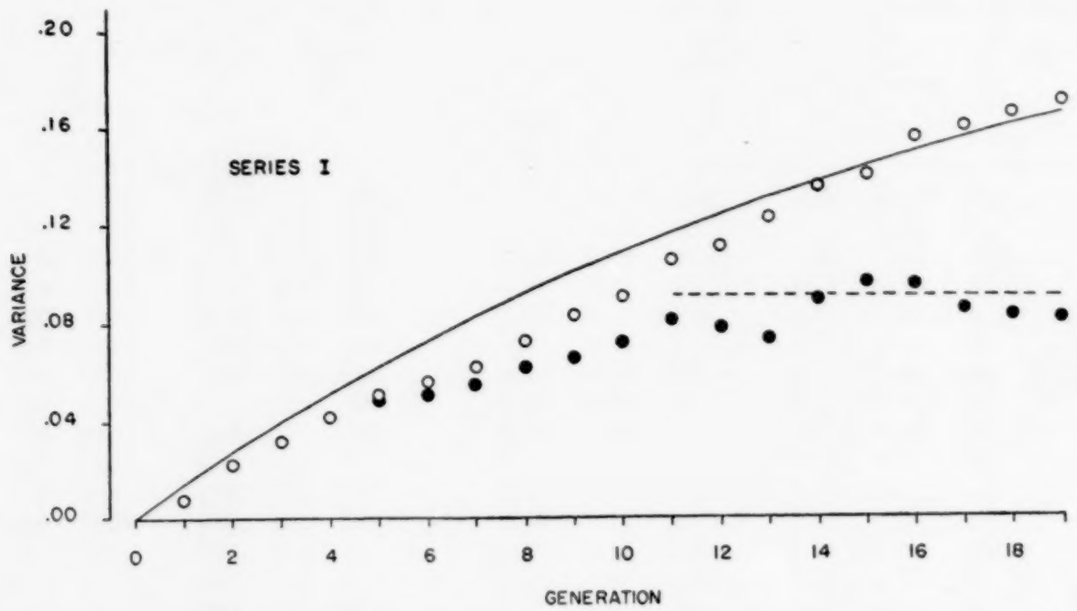

FIG. 12. Theoretical variances of the total frequency distribution by generation including fixed classes and based on a common estimate of $2N_e = 18$ for series I are represented by the smooth curves. Open circles show the observed variance of the distribution including previously fixed classes. Closed circles indicate the observed total variance excluding fixed classes. The asymptote (= 0.091) indicates approximately the theoretical maximum value of this variance. All values are on a relative scale.

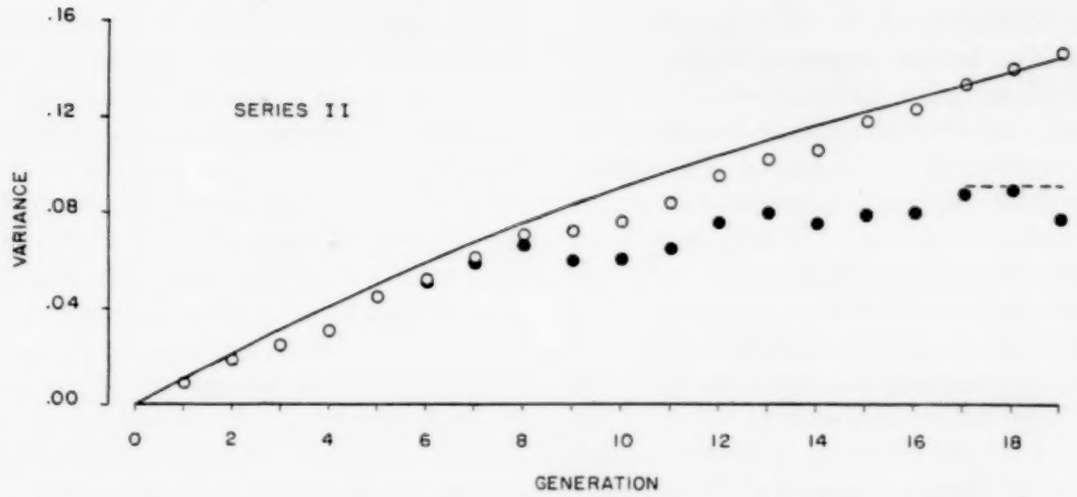

FIG. 13. Theoretical variances of the total frequency distribution by generation including fixed classes and based on a common estimate of $2N_e = 23$ for series II are represented by the smooth curve. Open circles show the observed variance of the distribution including previously fixed classes. Closed circles indicate the observed total variance excluding fixed classes. The asymptote ($= 0.091$) indicates approximately the theoretical maximum value of this variance. All values are on a relative scale.

assuming uniform frequencies for all classes has been accepted. This, again, should apply to the data of series I in the present case in spite of the non-uniformity of the theoretical distribution because of the closeness of the calculated distribution to linearity and the practical identity of the observed and theoretical mean frequencies. In computing the theoretical maximum variance excluding previously fixed classes no account has been taken of the slight falling off in frequency toward the extremes.

Table 17 and figures 12 and 13 present the observed and expected values of the variance on a relative scale by generation both excluding and including bottles previously fixed. The value of the theoretical maximum variance excluding previously fixed populations is in both cases $\simeq 0.091$.

The agreement of the data with expectation is fairly good in each case. Again, there is a closer similarity between observed and expected in series II. A greater stability is, of course, conferred on the observed values of the variance including fixed cultures in both cases by the fact that the proportion of freely varying components is gradually reduced as fixation proceeds.

The asymptote is approached by the observed variance excluding previously fixed classes in the 11th and 17th generations. This is in agreement with the apparent equilibrium of form of the frequency distributions in these generations of series I and II respectively.

DISCUSSION

The Random Drift Experiments

The evidence of heterogeneity among class estimates of N_e in series I apart from the significance of the regression of $1/2N_e$ estimates on class value is clearly related to the poor accord with expectation of the form of the terminal distributions of gene frequency in this experiment after stability of form had been approached.

The fact of a tendency toward higher recipient class variances in higher donor frequency classes can itself explain both the negative slope of the grouped frequency distribution over the terminal generations in series I and the equality of the fixation rate at the two extremes despite this slope. Both effects are due to a more rapid loss of cultures from the upper frequency range, either to fixation for bw^{75} or to classes of lower frequency.

But with regard to the secondary irregularities in the apparent form of the distributions after generation 11 in this case, it is of interest to consider the effect of variability in the value of $2N_e$ among the bottles of each generation on the stability of the total distribution. In theoretical considerations upon which it may be concluded that a stable form is approached in the distribution of gene frequencies among elements in a subdivided population, a constant value is assumed for the effective population size. In the absence of systematic pressures, each frequency class (q) contributes to the entire range of $2N + 1$ frequency classes according to the expansion of the binomial $[q(A) + (1 - q)(a)]^{2N}$ and to any particular class (q_1) in proportion to the $2Nq_1$th term of this expansion and to the frequency of donor class gene frequency (q) (Wright, 1931). Stability of form is reached when the rate of fixation is equal to $1/2N$ and the relative frequencies of the unfixed classes are constant and decaying at the rate of $1/2N$ per generation.

Variation in the effective value of $2N$ among the isolated components of a population would result in variation in the probability of a contribution being made by a given donor population at a frequency (q) to any particular recipient frequency class (q_1). Stability in the form of the distribution is dependent upon a proportional distribution of such probabilities from each donor class and upon the magnitude of the total frequency. Variability in the value of $2N$ and particularly gross heterogeneity in this factor would disturb the probability distribution among classes. Where the number of subdivisions is sufficiently large that each frequency class within the total range of unfixed classes from $1/2N$ to $(2N - 1)/2N$ is represented by a relatively large number of populations, no appreciable effect upon stability would be expected as the result of such heterogeneity. However, in cases such as are considered here, in which the mean frequency among unfixed classes during the last nine generations is less than three (series I), such a situation could bring about considerable distortion. These distortions would tend to compensate for one another within a series of generations and the grouped frequency distributions should more closely approach the theoretical form the larger the number of generations observed. But since a "condensation" in one part of the frequency range would not be expected to be immediately dissipated by a "rarefaction" in the same region, such compensation might easily require more than nine generations.

The somewhat better agreement of the observations with the theory in series II than in series I may be construed as rather direct evidence of greater heterogeneity in the average value of $2N_e$ among generations in the latter case. The differences between the results of series I and II are directly related to the size of the culture container in each case. The specific factor through which these differences are mediated is effective population size, but the immediate causes of reduction in the value of $2N_e$ as compared with the theoretical value in both cases, and the lower mean value, apparently greater variability, and functional relationship to q of $2N_e$ in series I, are not known. Variability in productivity extending to complete sterility is certainly a component. It seems probable that these effects are related to competition for oviposition space. In the vial cultures of series I the medium surface area was roughly three square centimeters as compared with about twelve for the series II containers. It is well known that a small environment can be quickly saturated with young larvae by a very few ovipositing females and the work of Sang (1949a, 1949b), although under quite different experimental conditions, showed that there is a strong relationship between larval mortality (as well as the duration of the larval period) and the degree of crowding of larvae. It was also found by Sang that successive batches of larvae

take longer to develop in more severely crowded cultures. It seems likely that the first females to oviposit would have a distinct advantage with respect to their contribution to the F_1 population, which would be the greater the more severe competition in the developmental phases became. However, there is no direct evidence in support of this conjecture under the conditions of these experiments.

The results of the present experiments may be compared with certain aspects of those of Kerr and Wright (1954a and b) on forked vs. type and spineless vs. aristapedia and Wright and Kerr (1954) on Bar vs. type. Selection was not altogether negligible in the case of forked, Bar was at a great selective disadvantage, and the heterozygote ss/ss^a was strongly favored, so the stable forms in these several cases were diverse. The agreement between observed and theoretical distributions of gene frequency was excellent in the two latter cases, comparable with that of series II of the present data, although based upon a much larger total frequency in one instance. None of the sort of disturbance which occurred in series I was apparent. The distribution could not be constructed in the case of forked because individuals were not completely classifiable according to genotype.

The cultures of Kerr's studies were maintained in vials of unspecified size with breeding populations of eight individuals as compared with sixteen in the present case. It seems likely that the degree of crowding was more closely comparable with that of the series II cultures than with series I, in accord with the evident association of crowding with excessive irregularity in the "stable" form of the frequency distribution in the present data.

With regard to estimates of effective population size, these authors found values relative to theoretical of 83% (forked), 72% (Bar), and 67% (spineless) best fitted the data. The last two values are in agreement with the estimate for series II (72%). The much lower average value obtaining in series I (56%) is, again, associated with severe crowding.

Crow (1954, 1955) has estimated the ratio N_e/N by quite different methods from those employed in population studies of the present type. He finds in experiments on *Drosophila melanogaster*, by a method involving enumeration of adult progeny, that for females $N_e/N = 0.71$ and for males 0.48. This suggests that in situations influenced equally by both sexes an intermediate value of about 0.60 might be expected, not far from the mean value for series I (0.56) of this study.

Selection

The alleles bw^{75} and bw have such a small difference in selective value as to escape detection in most aspects of these experiments. Changes in gene frequency attributable to a selective difference did occur, however, in three instances. But among these there is no agreement even as to sign and the effect in both the cage experiments and the series I random drift experiment was confined to the earliest generations. The data from the selection experiments were similarly inconclusive.

The association of genotypes having indeterminate differences in selective value with striking phenotypic differences seems to be unique among documented cases. Studies concerned with only a single character of selective value such as fecundity or only a part of the life cycle need not be considered in this context. The lowest known selective difference in the literature that has behaved with relative consistency under various conditions of continuous population culture is that between forked and type (Ludwin, 1951; Merrell, 1953; Kerr and Wright, 1954). However, even in this case considerable differences have been found under certain conditions and with a particular strain (author, unpublished). Ludwin (1951) found no evidence of significant changes in the frequency of raspberry over a period of more than six months in an array of cultures with an initial frequency of

0.50; however, Merrell (1953) reported a considerable differential, although under, again, quite different conditions of culture.

The experiments with bw^{75} vs. *bw* have so far included several different population densities ranging from the extreme of crowding in population cages to the conditions of the series II random drift experiments in which the number of parental flies per square centimeter of medium surface was approximately 1.25. All material was maintained at 25° C. and at high humidity. It may be that under different environmental conditions or against another "background genotype" definite differences in selective value can be found.

Summary

1) Competition between the alleles bw^{75} and *bw* in population cages gave no evidence of a trend in gene frequency change in any of seven cages during the period of observation, although the mean frequency over all cages at the time of the first sampling was significantly below 0.50, the frequency in the foundation flies.

2) Experiments designed to ascertain the relative viabilities and productivities of the several genotypes (bw^{75}/bw^{75}, bw^{75}/bw & bw/bw), male and female, showed significant departures from the expected segregation ratios in 3 of 21 population types. These differences could not be systematically interpreted and were not reproduced in a subsidiary study.

3) Study was made of the changes in the frequency of bw^{75} between generations in cultures perpetuated for 19 generations with random samples of 16 individuals. The sample size was sufficiently small to permit considerable random drift. Two series of about 100 cultures each were set up at an initial frequency of 0.50. Series I was conducted in 35 cc. homeopathic vials, series II in 60 cc. specimen jars. The total gene frequency in series I did not change systematically during the course of the experiment. In series II the gene frequency rose for four generations after which no consistent changes occurred.

4) The fixation rate after generation 11 in series I was not significantly different from uniformity by chi-square. Series II was not carried for a number of generations sufficient to permit a satisfactory estimate of fixation rate.

5) No evidence consistent with an hypothesis of selection appeared in either series when the sample frequencies were grouped according to donor frequency classes. From this standpoint gene frequency changes could safely be attributed wholly to accidents of sampling.

6) The distribution of gene frequency by generation among the bottles of series I and II approached stability of form in generations 11 and 17 respectively. The grouped frequency distribution over the last 9 generations in series I was in poor accord with an hypothesis of uniformity, based on the apparent absence of selection, showing both an excessive irregularity and a negative slope of probable significance. The grouped distribution over the last 3 generations of series II was in good accord with the expected uniformity.

7) Estimates of the average effective population size made from the observed recipient class variances gave values of N_e equal to 9.0 and 11.5 for series I and II respectively, 56% and 72% of the sample size of 16 individuals.

8) A highly significant regression of $1/2N_e$ on donor class frequency was observed in series I indicating higher variances at higher frequencies than could be accounted for on the assumption of a common estimate of effective population size. This effect explains the slope of the frequency distributions of series I after stability of form was approximated. The theoretical distribution on the assumption of the observed regression is in agreement with respect to general slope but indicates that stability of form had not been fully reached as early as preliminary considerations suggested. The lack of accord between the observed and theoretical distri-

butions of series I is discussed from several standpoints.

9) The observed fixation rate in series I was higher than expected assuming an effective population size of 9.0 but the difference was not significant.

10) Curves representing the expected increase in the total variance of gene frequency and decrease in the percentage heterozygosis over the period of observation were calculated for each series using the mean estimates of effective population size based upon observed recipient class variances. The agreement between the observed rates and rates indicated by the calculated curves was good in the case of series II but showed some inconsistency in series I.

11) The differences between series I and II with respect to effective population size estimates, dependence of $1/2N_e$ on q, agreement with expectation of the form of the distribution with expectation of the form of the distribution of gene frequencies after stability had been approached, the homogeneity of the observed recipient class variances, rates of increase in total variance and decrease in heterozygosis are related to the size of the culture container. The data of series I are more heterogeneous in all respects.

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A GENETIC APPROACH TO THE SYSTEMATICS OF PLANORBID MOLLUSCS

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The Problem

The present paper summarizes some observations on the Brazilian species of the molluscan family Planorbidae. The primary aim of the investigation has been to throw some light on the systematics of this family, which is still in a most controversial and confusing state. In fact, if the problem is approached with purely morphological methods, its solution is apparently an unattainable goal. Even a brief acquaintance with the pertinent literature will suffice to justify this statement.

Selfing and Crossing in Planorbid Molluscs

As far as we are aware, no previous attempt has been made to apply the biological species concept to the planorbids. The reason for this lies perhaps in technical difficulties. Planorbids are hermaphrodites capable of reproducing by self-fertilization. This makes it difficult at times to distinguish the offspring produced by crossing from that resulting from selfing. This difficulty was overcome by us at the very beginning of our studies. Having defined the two closely related allopatric species *Australorbis glabratus* and *A. nigricans* on morphological basis (Paraense and Deslandes, 1955a, b), we realized the necessity of excluding the possibility that the small differences observed between them might be mere expressions of intraspecific variation. It was obvious that difficulties of this kind would frequently recur in our subsequent work. Accordingly, we tried to find a way of performing breeding experiments which could make our judgments concerning the specific characterization of the planorbids reasonably reliable.

Previous studies by several authors have shown that several planorbids are capable of reproducing by self-fertilization when grown in isolation. The writer has confirmed this for *A. glabratus* (Paraense, 1955), and for other species of *Australorbis* subsequently studied (*nigricans, centimetralis, janeirensis,* etc.). Owing to the faculty of self-fertilization, the reproductive activity of the snails is unimpaired by keeping individual snails in isolation. However, since specimens in copulation are frequently observed in the colonies, the next step was to ascertain the extent of utilization by a snail of the own and foreign sperm under natural conditions. In this study the writer has made use of the factor of total albinism. The albinism is determined by a single recessive gene, and therefore can be used as a convenient genetic marker (Paraense, 1955). It may be noted that the total albinism, in which there is no development of the eye spots, differs from partial albinism; in the latter the eyes are pigmented and the character is polygenic. In both kinds of albinism the body of the animal is red, due to the presence of hemoglobin in the blood.

Since a total albino is homozygous for a recessive gene, it must produce exclusively albinos by self-fertilization. If mated to a homozygous pigmented conspecific individual, an albino will produce pigmented offspring (owing to the dominance of the normal allele) when cross-fertilization takes place, or albino offspring in case of self-fertilization. Production of both albino and pigmented individuals obviously indicates simultaneous occurrence of some self- and some cross-fertilizations. A distinction between cross- and self-fer-

tilized progenies may easily be made already in intracapsular embryos, because of the presence or absence of the eye spots at the base of the antennae; when present, they are clearly visible in six days old embryos grown at about 22° C.

An albino individual which had produced a progeny by self-fertilization may subsequently be mated to a pigmented individual. If the pigmented individual belongs to the same species and geographic race as the albino, the latter begins very soon to produce a pigmented progeny resulting obviously from cross-fertilization. Cross-fertilization continues as long as the specimens remain together. If after this the members of the couple are separated, the albino continues producing pigmented offspring for a time varying from about 20 days to two months or even more. This indicates that the foreign spermatozoa introduced during copulation into the genital tract of a snail are stored up for subsequent utilization. The place for such storage seems not to be the so called "spermatheca," the function of which is still unknown. It is probable that the foreign spermatozoa are stored up in the "seminal vesicle" (a convoluted swelling of the hermaphroditic duct), which is traversed by the ova and in which the own spermatozoa also accumulate.

That, in their natural environments, the planorbids normally reproduce by cross-fertilization is demonstrated by the fact that, for a relatively long time after being brought to the laboratory, the albinos collected in nature deposit eggs which almost always develop into pigmented embryos. Pigmented embryos come from fertilizations by spermatozoa of homozygous pigmented specimens, which are most frequent in natural populations.

In planorbids, the ova and the spermatozoa are formed side by side in the same epithelial walls of the ovotestis. They pass together into the lumen of the ovotestis, and travel together down the hermaphroditic duct. This arrangement might seem to lead to self-fertilization. And indeed, the eggs and the sperms of an isolated individual readily unite and produce viable embryos. And yet, they become reciprocally indifferent as soon as the spermatozoa from another conspecific individual are introduced by copulation. Spermatozoa of another individual enjoy an absolute preference as fertilizing agents. This phenomenon of selective fertilization was studied in detail in *Bulinus contortus* by Larambergue (1939). The explanation of this phenomenon is not clear. However, its evolutionary significance is worthy of being emphasized. Because of it, a population secures its own preservation through gene flow and recombinations, which helps it to maintain a store of genic variability. Thus, in spite of the planorbids being hermaphroditic organisms perfectly capable of self-fertilizing, their colonies must be regarded as Mendelian populations in the same sense as those of dioecious organisms. This conclusion has far-reaching implications, insofar as it permits, regarding the planorbid populations as evolutionary units subject to the same rules governing the evolution of Mendelian populations in general (see Dobzhansky, 1950).

Failure of Interspecific Crosses

Once acquainted with the facts reviewed above, we were prepared to perform and interpret the results of breeding experiments between species and races of planorbids. Such experiments were first made using pigmented *A. glabratus* and albino *A. nigricans,* or vice-versa. The two forms proved to be completely reproductively isolated (Paraense and Deslandes, 1955c). Similar experiments were also made between the two species named above and *A. centimetralis* (Paraense and Deslandes, 1955d). No hybrids were obtained. In all of such tests the albino and pigmented specimens of each couple maintained themselves in strict reproductive isolation, each member of the pair giving origin separately to its own offspring exclusively by self-fertilization.

Unpublished results of a large number of tests between the above mentioned and other planorbids (*Australorbis* sp., *A. janeirensis, Helisoma* sp.) have given similar negative results. Until now we have obtained not a single hybrid between these species.

The systematics of the planorbids may now be established on a solid biological basis. The main difficulties to be dealt with now center around the question of the taxonomic value of morphological characters. As an example, we may consider the renal ridge in *A. glabratus*. This is a medial fold running along the membrane which covers ventrally the renal tube. All the large planorbids so far met with by us in this country are separable into but two forms—one with, the other without, the ridge. The genitalia of both forms show no appreciable difference. The shell characters, allthough allowing separation of typical specimens, show much intergradation. The question then arises whether the only observed qualitative difference, the renal ridge, can be relied upon to differentiate the species. The answer was given by breeding experiments. Specimens with and without the renal ridge never produced hybrid progenies. It is reasonable to regard them as specifically distinct. In turn, all the populations of ridged individuals (*A. glabratus*) are interfertile, as well as the unridged ones (*A. nigricans*). In the same way, it is possible to test the taxonomic value of the other characters. The fact that the two just mentioned species are morphologically separable by only a single character has led many workers to confuse them under the name *A. glabratus*. Indeed, if the character of the renal region is neglected (as it frequently has been) they will look to the systematist as true sibling species.

The genetic method has recently been applied (Paraense and Deslandes, 1956) in a study of the planorbids from Santos, State of São Paulo. The specific identity of these snails has been subject to much controversy. They have been classified under six different names corresponding to five distinct species, including two which are restricted to the African continent. We showed that all the populations studied were conspecific and identical with *A. nigricans,* since they freely hybridized with a geographically distant population of the latter species.

A better understanding of the evolutionary processes in the planorbid family is to be expected from breeding experiments on its members. Our observations on this subject are still in exploratory stages, but it is obvious that a wide field of productive work is open.

Crosses of Conspecific Allopatric Populations

Breeding experiments between planorbids from allopatric conspecific populations have yielded some interesting results concerning the attainment of different degrees of incipient reproductive isolation between such populations. Specimens of *A. glabratus* from Santa Luzia (Minas Gerais) and from Estremoz Lake (Rio Grande do Norte), localities about 1900 Km apart, readily produced fertile hybrids in the laboratory. However, the events observed in these experiments were somewhat different from those recorded above when dealing with individuals from the same population. In the latter case, as stated above, cross-fertilization is steadily maintained as long as the specimens remain together. It continues also for a time after the separation of the couple, until exhaustion of the stored foreign spermatozoa. When individuals from remote populations are mated, cross-fertilization never completely replaces self-fertilization. With few exceptions, all the egg-masses from the albinos showed a mingling of pigmented and albino embryos, thus indicating some instability in the cross-fertilizing mechanism. In addition, separation of the couple is followed by a relatively speedy disappearance of the pigmented embryos, owing perhaps to a

rapid exhaustion or incapacitation of the stored foreign spermatozoa.

These results indicate, in a rather unique way, a lowered fertility between allopatric conspecific populations of *A. glabratus* as a result of geographic isolation. Among dioecious organisms fertility of crosses may be expressed sequentially by the degrees of hybrid viability, hybrid fertility and hybrid breakdown. In our experiments with the hermaphrodite *A. glabratus* the numbers of surviving hybrids in the interpopulational crosses were widely variable, but no significant differences compared to intrapopulational crosses were observed. Moreover, hybrid fertility was apparently equivalent in both cases. As to the F_2 hybrids, we have observed a wide variation in viability, but so far no observations on hybrid breakdown during post-embryonic life have been made. Variations in the degree of viability may be due to inbreeding rather than to genetic incompatibility, since the experiments involve some self-fertilized progenies. The lowered fertility of matings between geographically distant conspecific populations appears to be due to the failures of foreign spermatozoa to fertilize all the available eggs. It seems evident that the partial intersterility depends in these cases on a certain degree of gametic isolation. This isolation is caused perhaps by some deleterious effects of the genitalic secretions, or by some other biochemical response against foreign spermatozoa. Be that as it may, we are dealing here with an instance of the development of a degree of reproductive isolation between allopatric populations. It is interesting to note how self-fertilization makes up for the deficiency in the mechanism of cross-fertilization.

Results similar to the above were obtained also in crosses between snails from Santa Luzia (Minas Gerais) and from Recife (State of Pernambuco), separated by a distance of about 1700 Km. An apparent attainment of the stage of virtually complete reproductive isolation was observed between *A. glabratus* from Santa Luzia and from João Pessoa (State of Paraiba). These localities are about 1800 Km apart, João Pessoa being situated midway between Estremoz and Recife. The albino members of fifteen couples laid 1151 eggs, of which 116 were inviable. Of the viable ones, only 12 produced pigmented (hybrid) embryos, the remaining 1023 being albinos. The hybrids came from only two couples, the other thirteen couples have proved intersterile.

Crosses between the snails from João Pessoa and from Recife (about 100 Km apart) are being now attempted; of the first five couples thus far observed, only two produced a relatively small proportion of hybrids. These initial results show that the population from João Pessoa is a distinct local race of *A. glabratus*.

The fact that allopatric conspecific populations of planorbids develop various degrees of reproductive isolation emphasizes the role of geographic speciation in the evolution of this animal group. If in these molluscs the Mendelian populations are evolutionary units, and if geographic speciation plays a prominent role in their evolution, it must be expected that the evolutionary patterns in the planorbids will not differ substantially from those in other better-studied groups of cross-fertilizing organisms. It should not be forgotten, however, that the self-fertilization may conceivably lead to the occurrence of sympatric speciation. This possibility should not be neglected in future studies on the evolution of the planorbids.

Summary

Recessive albino mutants occur in natural populations of several species of planorbid molluscs. These mutants have been used as genetic markers in experiments planned to place the systematics of this family on a sound biological basis. These experiments have yielded the following general conclusions.

1. A snail grown in isolation freely reproduces by self-fertilization, but this process is readily and completely replaced by cross-fertilization if the snail is placed

with another conspecific individual from the same population.

2. Cross-fertilization does not entirely replace self-fertilization in mates between conspecific allopatric individuals. Such crosses result in a lowered production of hybrid offspring. However, the fertility of the snail is kept unimpaired, owing to self-fertilization of those eggs which failed to be cross-fertilized. This fact indicates that the lowered interfertility depends on variable degrees of gametic isolation rather than on any cytogenetic mechanisms.

3. All the recognizable morphological species so far studied by us have shown absolute reproductive isolation.

4. In spite of the planorbids being hermaphrodites perfectly capable of self-fertilizing, their colonies must be regarded as Mendelian populations in the same sense as those of dioecious organisms.

5. It seems that geographic speciation is paramount also in the evolution of the planorbids. However, it is possible that the faculty of self-fertilization may favor in some special cases also sympatric speciation.

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ADDITIONAL OBSERVATIONS ON CHROMOSOMAL TRANSLOCATIONS IN COTTON HYBRIDS

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The chromosomes of some plant genera abound with segmental interchanges but in the genus Gossypium only three have been found thus far. In a previous study, hybrids between the two Old World species *G. herbaceum* and *G. arboreum* formed one group of four catenated chromosomes at meiosis whereas hybrids between the New World cotton *G. hirsutum* and *G. herbaceum* had two such groups. Three different reciprocal translocations produced these rings or chains of four as was proven by the behavior of hybrids between *G. hirsutum* and *G. arboreum* which exhibited one ring of four and one of six chromosomes in most pollen mother cells. Of particular interest was the constitution of an Old World species known only from the wild, *G. anomalum*, which had the arrangement of *G. herbaceum* with respect to visible interchanges. *G. anomalum* × *herbaceum* hybrids produced no ring configuration while hybrids between *G. anomalum* and *G. arboreum* had one group of four (Gerstel, 1953).

These results strengthened the viewpoint (Hutchinson, 1954) that *G. herbaceum* was the more primitive of the cultivated Old World species and also suggested that it was closer than *G. arboreum* to the phylogenetic route which led from the Old World to the cultivated New World cottons. The data had two gaps. First, the strains of *G. arboreum* studied were largely northern annuals and advanced types from China, Pakistan and Burma; the more primitive and *herbaceum*-like (Silow, 1944) race *indicum* was not represented nor was race *soudanense* from Africa. It was quite possible that inclusion of the latter races might have changed the picture. Furthermore, only one of the two cultivated species of the New World had been included. To close these gaps, we will broaden the range of *G. arboreum* types in this paper and will add *G. barbadense*, the second New World cultivated species and also *G. tomentosum* from Hawaii.

Plant Materials

This study would not have been possible but for the generous gifts of seeds from various quarters of the globe. Lines of *G. arboreum* race *indicum* were supplied through the courtesy of Dr. J. B. Hutchinson of the Empire Cotton Corporation. Below are listed the accession numbers under which the seeds were received, their common designations and Dr. Hutchinson's description:

ATI 1	Humpi	"Primitive perennial, secondarily wild from the Deccan, Peninsular India."
ATI 2	Nadam	"Cultivated perennial from Northern Madras."
ATI 5	Coconadas	"Cultivated perennial from Northern Madras, agriculturally superior to Nadam."
ANI 17	Gaorani 6	"Cultivated annual from Hyderabad, Deccan."

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These descriptions indicate that the four strains represent a series in the development of the *indicum* cottons.

ANI 11, 11a, 11b, 11c Karakhadi — "These were selections for wilt resistance made in a part of Baroda State where Rozi (*arboreum*) and Goghari (*herbaceum* race *wightianum*) are grown mixed. They are *arboreums*, but look as if there had been introgression from *herbaceum*."

Seeds of race *soudanense* (accession ATS 29, Empire Cotton Corporation) were kindly supplied by Mr. R. L. Cuany.

Each of these new materials was crossed with known tester lines of *G. arboreum* and *G. herbaceum*. The testers, listed in the footnote to table 1, had the typical chromosome arrangements of their species as previously ascertained (Gerstel, 1953).

As representatives of *G. barbadense*, we chose varieties which were reputedly free from introgression from *G. hirsutum*. One such variety was obtained from Mr. J. G. Jenkins from Georgia under the name of Sea Island TZVR. The remainder were placed at our disposal through the good offices of Dr. W. Wouters of the Institut National pour l'Étude Agronomique du Congo Belge. They represent the four main types of the species found in West Africa. For the history of their introduction by the slave trade and present distribution, the reader is referred to publications by Wouters (1948) and Hutchinson (1949). These lines are

1) *brasiliense* (naked seed kidney type)
2) *peruvianum*
3) *barbadense sensu strictu*
4) *vitifolium*.

1) to 3) were described by Dr. Wouters as native primitive dooryard cottons of the Congo. He suspected that the sample of 3) may have had some introgression from *peruvianum*. Type 4) is a more modern selection, called Ishan, and shows some visible effects of past hybridization with *G. hirsutum*. It was used only because no pure *vitifolium* was available.

Two lines of the Hawaiian *G. tomentosum* were employed. One was derived from seed which had originally been obtained in 1948 from the collection of the Empire Cotton Corporation as accession TP9-3 and the other was collected more recently in Hawaii by Dr. A. J. Mangelsdorf.

As test plants from *G. hirsutum*, two lines were used: Coker 100 W, a standard commercial variety and S4025, a synthetic genetical marker strain.

Experiments

In the case of the Old World species, small plantings were made in the greenhouse from the seeds received from abroad and one plant, or sometimes two different ones, from each accession were crossed with both *G. herbaceum* and *G. arboreum*. Pollen mother cells were gathered from usually two progeny plants of each of these crosses and prepared as described by Gerstel (1953). If catenations were found, their presence was ascertained in at least five, but usually more cells from each plant. In table 1, an entry "11 II + 1 IV" thus denotes that a chain or ring of four chromosomes, plus 11 pairs, were found in at least 10 cells coming from two different F_1 plants. We tried to decide only whether a given plant was a structural hybrid or not and made no attempt at evaluating the frequency of occurrence of IV's as had been done in the earlier paper. There it was stated that about 88% of the PMC's of *G. arboreum* × *herbaceum* hybrids had a chain or ring of four chromosomes.

When catenations were not apparent, then at least ten well-spread cells of usually two sister plants were analysed be-

TABLE 1. *Metaphase conjugation in Old World hybrids* *

Tested lines	× *herbaceum*	× *arboreum*
Indicum:		
Humpi	11 II + 1 IV	13 II
Nadam	11 II + 1 IV	13 II
Coconadas	11 II + 1 IV	13 II
Gaorani	11 II + 1 IV	13 II†
Karakhadi 11	11 II + 1 IV	13 II
Karakhadi 11a	13 II (1 plant)	11 II + 1 IV (2 plants) 13 II (1 plant)
Karakhadi 11b	11 II + 1 IV	13 II
Karakhadi 11c	11 II + 1 IV	
soudanense:		
ATS 29	11 II + 1 IV	13 II†

* Only maximum conjugation is listed. *Herbaceum* testers used were P.E.I. 173 703 and HTP 8; *arboreum* tester was Sanguineum for all lines but Karakhadi 11b. These testers were described previously (Gerstel, 1953) as having the type arrangements. Karakhadi 11b was tested with S4065, a synthetic genetic strain of *arboreum* of northern type.

† Only one hybrid tested.

fore an entry was made in table 1. Overlapping pairs of chromosomes were sometimes difficult to distinguish from translocation figures and where they occurred, additional cells were studied to ascertain that structural heterozygosity was not present.

The result of these studies, as compiled in table 1, is that all *indicum* and *soudanense* lines, with one exception, differed from the *G. herbaceum* testers by one translocation and had the segmental arrangement which had been found previously typical of the more northern *arboreums*. The one exception was Karakhadi 11a which had undergone introgression from *G. herbaceum* as mentioned above. In this particular case, a single Karakhadi 11a plant had been crossed with both *herbaceum* and *arboreum* testers; one offspring from the former and three from the latter cross were analysed. Of these four hybrids, three had been produced by Karakhadi 11a gametes with the *herbaceum* pattern, and one by a gamete with the *arboreum* pattern; therefore, Karakhadi 11a apparently was a structural hybrid.

The tetraploid New World species, *G. barbadense* and *G. tomentosum*, were tested by crossing single representatives of the various accessions with *G. hirsutum* or with each other and two hybrids of each cross planted in the open. At least fifty PMC's of each cross were analysed for the presence of multivalents; approximately equal numbers were obtained from

FIG. 1. Metaphase of *G. barbadense* race *vitifolium* × *hirsutum* with 26 ring bivalents.

FIG. 2. Metaphase of *G. barbadense* TZVR × *arboreum* with 8 II + 1 IV + 1 VI + 13 I.

FIG. 3. Metaphase of three-species hybrid 4n (*arboreum* × *thurberi*) × *barbadense* TZVR with 18 II + 1 VI + 1 VIII + 2 I.

(All three figures are camera lucida drawings to the same scale as indicated on figure 1.)

TABLE 2. *Configurations in pollen mother cells of New World hybrids*

Hybrids	*barbadense* ×*tomentosum*	*hirsutum* ×*tomentosum*		*barbadense* ×*hirsutum*				
Strains	TZVR × TP 9–3	Coker × Mangelsdorf Collection	S4025 × TP 9–3	*brasiliense* ×Coker	*peruvianum* ×Coker	*Afric. barb.* ×Coker	*vitifolium* ×Coker	TZVR × S4025
26 II†	55*	44	54(3)	44	48(1)*,**	100	39*,**	37(1)*
24 II + 1 IV	1	2	0	2	1	1	0	2
Overlaps	14	5	2	7	7	22	12	18
Total PMC's	70	51	56	53	56	123	51	57

† Numbers in parenthesis indicate PMC's with 25 II + 2 I.
* 1 PMC had only 1 open bivalent.
** 1 PMC had only ring bivalents.

each of the two sibs, and the results were combined in table 2. Figures of four conjugated chromosomes occurred in the metaphases of most hybrids, but only rarely, and their significance will be discussed. Most cells contained 26 pairs. In this material with a higher chromosome number, it was sometimes not possible to determine whether certain groups of chromosomes represented multivalents or merely overlapping bivalents. Therefore, it was necessary to insert in table 2 a special column headed "Overlaps" in which were listed cells with one or more uncertainties. Probably very few, if any, were actual multivalent configurations.

Attention was paid to cells containing 25 or 26 closed ring bivalents. Such were found in a few instances (fig. 1) and registered in table 2. Their occurrence represented positive evidence that translocations of entire arms were absent. (A case against the presence of interchanges of terminal segments could hardly be made by this method where interstitial chiasmata occur as in most of our cells (fig. 1); obviously interstitial chiasmata could be located proximally of the points of interchange. In the absence of observed interstitial chiasmata, the argument against translocations would be based on the assumption that terminalization of chiasmata in interstitial segments beyond points of interchange does not occur; this has been asserted to be the case by Darlington (1937) but was disputed by Gottschalk (1954) and others. However, it is most likely that the translocations in the A genome which were under consideration involved entire arms or their major portion because they caused catenation in most PMC's of the known structural hybrids.)

Positive evidence could also be obtained that the A genome of *G. barbadense* is similar to that of *G. hirsutum* because a *barbadense* × *arboreum* hybrid gave a configuration of six and one of four chromosomes like the *hirsutum* × *arboreum* hybrids studied before. The majority of PMC's from *barbadense* TZVR × *arboreum* contained 8 II + 1 IV + 1 VI + 13 I (table 3; fig. 2). The approach was not extended to the remaining *barbadense* races because of the difficulty with which New World × Old World hybrids are produced. The use of three-species hybrids 4n (*thurberi* × *arboreum*) × *barbadense* was considered, but conjugation between homeologous chromosomes of the A and D genomes was so frequent as to

TABLE 3. *Metaphase conjugation in* G. barbadense *(TZVR)* × arboreum *(S4065)*

	Number of PMC's
8 II + 1 IV + 1 VI + 13 I	13*
7 II + 1 III + 1 IV + 1 VI + 12 I	1
7 II + 1 IV + 1 VI + 15 I	1
7 II + 1 IV + 1 IV(?) + 1 VI + 11 I	1
9 II + 1 III + 1 VI + 12 I	1
9 II + 1 IV + 1 VI + 11 I	1 + 1(?)
10 II + 1 VI + 13 I	3
Total	22

* Includes one PMC with a missing univalent.

obscure the issue. Three clear cells from such a hybrid had the following configurations: 17 II + 2 IV + 1 VI + 4 I, 20 II + 1 IV + 1 VI + 2 I and 18 II + 1 VI + 1 VIII + 2 I (fig. 3). The occurrence of multivalents involving homeologous chromosomes in three-species hybrids between *G. hirsutum* and *G. thurberi* × *arboreum* amphiploids has been noted earlier by Menzel and Brown (1954) and was discussed by those authors.

Discussion

The results demonstrate even more forcibly than the previous paper the rarity of segmental interchanges in the genus. Pending the outcome of additional tests of two very primitive perennial arboreums not yet studied (Rozi from Gujerat and a type from Madagascar), it appears that *G. arboreum* is uniform throughout its range with respect to the characteristic under study. Karakhadi 11a is the exception which confirms the rule because of introgression from *G. herbaceum* which was mentioned above. As a morphological manifestation of this, our plants of Karakhadi 11a had reflexed bracteoles and round bolls which were an obvious indication of influence of *G. herbaceum*.

Hybrids between *G. hirsutum* and *barbadense* had been studied cytologically before. Webber (1935, 1939) and others observed fairly normal pairing relations (s. below). But most varieties of *barbadense* from the United States may be suspected of introgression from Upland (*G. hirsutum*) and are, therefore, no reliable indicators. The new results from hybrids with a variety of presumably *hirsutum*-free *barbadense* lines show that the two species, and also *G. tomentosum*, were alike with respect to the structural combinations under investigation. Different arrangements of the segments concerned would have produced multivalent configurations in the majority of cells as has been experienced previously (*loc. cit.*). Positive proof was also adduced by the *G. barbadense* × *arboreum* hybrid (table 3) which showed the same configurations described for *G. hirsutum* × *arboreum* and which indicated that three translocations distinguished the chromosomes of the Asiatic species from the American amphiploids. The behavior of our *G. barbadense* × *arboreum* is in substantial agreement with that of a similar one studied by Skovsted (1934). Beasley (1942) had eight cells of a hybrid between Pima (*barbadense*) and *G. herbaceum;* four of these contained two quadrivalents each, but in one Beasley saw a hexavalent which is unexpected.

We found occasional multivalents in most hybrids between the amphiploid species. Similar observations had been made by Webber (1935, 1939) who saw occasionally as many as three quadrivalents on a cell plate and by Beasley (1942). These authors found some multivalents also in the pure amphiploid species (Webber, 1934, 1938; Beasley, 1942), but they are apparently less frequent there than in hybrids. Menzel and Brown (1954), especially, emphasize that in pure *G. hirsutum* "no convincing cases (of multivalents) have been seen in this laboratory among several thousand pollen mother cells examined each year." The multivalent associations which we detected in the amphiploid hybrids could be due to short interchromosomal exchanges differentiating the species and permitting formation of an occasional chiasma in hybrids. The second possibility should be considered that the factors which control strictly preferential pairing between homologues in an allopolyploid species are upset in interspecific hybrids thus permitting an occasional chiasma to form between homeologous segments of chromosomes belonging to different subgenomes. As a third possibility, one cannot discount at the present time that the evolutionary changes which occurred in the chromosomes to make possible preferential associations proceeded in opposite directions in different amphiploids such that segments of a given A chromosome of *hirsutum* have retained a closer affinity

to segments of the D homeologue of *barbadense* than to the A homologue of the latter species. The frequencies of metaphase association between such segments would depend on their length.

A consideration of the distribution of translocations in the genus indicates that they are limited to the A genome of the cultivated species of the Old World and of the New World amphiploids. These species with the exception of the Hawaiian *G. tomentosum* are cultigens covering wide territories. No translocations have been found either in hybrids between D-species nor in hybrids between the latter and the amphiploids (see Brown and Menzel, 1952, for references). Douwes (1953) has recently studied pairing behavior in hybrids between wild species of the other sections. He saw only bivalent pairing in *G. anomalum* × *triphyllum* (B-genomes). Similarly, he gave no evidence of translocations in the three species with E-genomes, *G. somalense*, *G. areysianum* and *G. stocksii*. Douwes' work completed study of all intrasectional hybrids possible now. The only intersectional hybrids between diploid species with a chiasma frequency sufficient for multivalent formation are those involving the A and B-genomes; as stated in the introduction B-genome *G. anomalum* has an arrangement similar to that of *G. herbaceum*.

Any explanation why the only translocations known in the genus are limited to one genome of the cultigens (disregarding *G. tomentosum*) must remain purely speculative.

It is tempting to exploit the A-genome translocation for the establishment of phylogenetic relationships, even though reasoning based on a single characteristic is weak. Similarity of segmental arrangements of chromosomes has been used as an indication of relationship in other genera (e.g., Cleland, Preer, and Geckler, 1950), but with the argument that "similarities (in structure) are too frequent to be explicable in terms of chance." Any argument for the phylogenetic significance of the A-genome translocations in our case could be based, in contrast, only on the rarity with which they occur in Gossypium and that a reversal of a particular translocation is, therefore, not a likely event. Thus, a phylogenetic pathway from *G. anomalum* to *G. herbaceum* and thence to the New World species has been suggested (Gerstel, 1953). In this scheme, *G. arboreum* would lie off the main route, because it is distinguished by an additional translocation from both *G. herbaceum* and the American amphiploids.

The problem of a mono- *vs.* a polyphyletic origin of the three amphiploid species remains unsolved, except that all three have a segmental arrangement similar to that of *G. herbaceum*. Morphological characters, however, tell a different story: Wouters (1948) has commented on the striking similarity with respect to capsule characters between *G. arboreum* and *G. barbadense* on the one hand and *G. herbaceum* and *G. hirsutum* on the other. The future taxonomy of the genus will have to account for both sets of observations.

Summary

1. The previous investigation of chromosomal arrangements in Old World cottons was extended to include races *indicum* and *soudanense* of *G. arboreum* with the result that all representatives of this species carry the same chromosomal arrangement and differ from *G. herbaceum* by one major segmental interchange.

2. The three Gossypium species of the New World were found not to differ by any segmental interchange involving a major portion of a chromosome arm. They are closer to the *G. herbaceum* than the *G. arboreum* arrangement.

3. The results suggest *G. herbaceum* as a common ancestor of all three New World species but are by no means conclusive.

4. Possible reasons are discussed why multivalent formation between homeologous chromosomes may be more frequent in interspecific hybrids of New World species than in pure species.

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INTERSPECIFIC HYBRIDS IN NEW ZEALAND *AGROPYRON*

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Introduction

In a previous paper (Connor, 1954) descriptions and distribution of the four indigenous species of *Agropyron* were presented along with descriptions and distribution of infraspecific units in *A. scabrum*, the breeding systems of the species were considered, and some data on interspecific hybridization were presented. This paper reports results from further work on interspecific hybrids in New Zealand members of the genus, and attempts to define the biosystematic status of the four species. Finally, the taxonomic treatment of the species is related to the biosystematic status.

As previously reported (*op. cit.*) spontaneous interspecific hybridization [1] in experimental gardens is not uncommon among native species of *Agropyron*, but natural hybridization is not commonly observed. Hybrids which have been detected by morphological studies and verified by chromosome counts are:

A. scabrum (n = 21) ♀ × *A. kirkii* (n = 21) ♂ and reciprocal

A. scabrum (n = 21) ♀ × *A. tenue* (n = 28) ♂

A. enysii (n = 14) ♀ × *A. scabrum* (n = 21) ♂

A. enysii (n = 14) ♀ × *A. tenue* (n = 28) ♂

J. B. Hair (pers. comm.) reports that for cytological work on New Zealand *Agropyron* whilst at The John Innes Horticultural Institution he artificially produced interspecific hybrids of all six possible combinations. Numerous artificial crosses have been made between *A. scabrum* and *A. kirkii*, and artificial backcrosses produced. Infraspecific units in *A. scabrum* which were described in 1954 were called Group Otago, Group Teviot etc. but for simplicity these are referred to here as "Otago," "Teviot," etc.

Fertility of Hybrids

The fertility of hybrids is one important indicator of biosystematic relationship, for it may be a measure of the ability of the species to exchange genes. To give an estimate of the amount of gene exchange possible between species, both pollen and seed fertility of the hybrids have been determined.

A. kirkii × *A. scabrum* (2n = 42 [2])

To determine the percentage of good pollen grains in F_1 *A. kirkii* × *A. scabrum* pollen grains were mounted and stained in a mixture of methyl green, phloxine and glycerine jelly, (Owczarzak, 1952) and examined; the percentage of stained, well filled grains in some F_1 hybrids is shown in table 1.

Numerous tests to determine the self-fertility of this hexaploid hybrid combination have been made. The greatest seed-set obtained from selfing was 6.5%, but most selfings of the F_1 yield less than 1% of filled florets, percentages such as 0.2; 0.3; 0.4; 0.6 and frequently 0.0 indicating the low self-fertility. When F_1 hybrids are open-pollinated there is a greater seed-set than under selfing. Table 2 showing three examples from an F_1 of *A. scabrum* "Otago" ♀ × *A. kirkii* ♂ indicates the difference between self- and open-pollination. In these cases the seed-set under open-

[1] Spontaneous hybrids are those which arise in experimental gardens, from uncontrolled hybridization.

[2] Chromosome numbers here and in later cases determined by Dr. J. B. Hair.

TABLE 1. *Percentages of good pollen grains in* F_1 A. kirkii × A. scabrum

F_1			% Good pollen
A. scabrum	"Otago"	× *A. kirkii*	18.0
	"Wellington I"	× *A. kirkii*	48.1
	"Wellington II"	× *A. kirkii*	29.8
	"Molesworth"	× *A. kirkii*	49.6
	"Tekapo I"	× *A. kirkii*	40.7
	"Teviot"	× *A. kirkii*	14.1

pollination was mostly from backcrossing to the male parent *A. kirkii* or from F_1 intercrossing. Both parental species were present in the block in which the hybrids were growing. Some of the difference between open- and self-pollination can be attributed to the effects of bagging, but the progeny raised from open-pollination indicated that back-crossing accounted for most of the difference.

A similar difference between self- and open-pollination occurred in an F_1 *A. scabrum* "Wellington I" ♀ × *A. kirkii* ♂; but a progeny raised from an open-pollinated F_1 plant showed a pronounced increase in fertility under both self- and open- pollination. Under selfing, the F_1 set 0.2% seed while under open-pollination 4.2% seed was set. One plant raised from the open-pollinated seed, set under selfing 30.8% seed, and under open- pollination 35.7% seed, which values are not far below those frequently found for open- or self-pollination in *A. scabrum* "Wellington I."

The results obtained from self-pollinating F_1 plants indicate that there is only a very limited amount of direct gene exchange possible between *A. kirkii* and *A. scabrum*. When F_1 hybrids have arisen there is, however, a greater chance for gene exchange through back-crossing or free inter-crossing among F_1 hybrids. It has been shown that in *A. scabrum* "Wellington I" ♀ × *A. kirkii* ♂ there was a pronounced recovery in self-fertility following open pollination (where back-crossing to either parent or free F_1 inter-pollination could occur). Progenies raised from open-pollinated F_1 hybrids indicated that backcrossing had taken place.

TABLE 2. *Percentage seed-set in 3 plants of* F_1 A. scabrum "Otago" ♀ × A. kirkii ♂

Plant No.	Percentage seed-set	
	Self-pollination	Open-pollination
3028	0.3	6.7
3029	0.5	8.2
3030	1.3	6.1

During the season 1950/51 four plants of an F_2 *A. scabrum* "Otago" ♀ × *A. kirkii* ♂ were self-pollinated, but no seed was set in 930 florets. In the following season a further attempt was made on the same plants to produce seed for an F_3, but no seed was set in 392 florets. No seed was set under open-pollination in either year, although there were some few well-filled pollen grains in a sample from this F_2 progeny.

In general, though many attempts have been made to obtain F_2 generation *A. kirkii* × *A. scabrum,* the low fertility of the F_1 hybrids appears to be a serious limiting factor; the only F_2 generation raised was from *A. scabrum* "Otago" ♀ × *A. kirkii* ♂ mentioned above.

A. scabrum ♀ × *A. tenue* ♂ (2n = 49)

This combination occurred once in the experimental garden, and is known only with *A. scabrum* "Wellington I" as the female parent. The single plant is vigorous, and has been increased by division. No seed has set either under self- or open-pollination, but there was 2.0% good pollen. This hybrid is heptaploid, and sterile, with gene exchange improbable.

A. enysii ♀ × *A. tenue* ♂ (2n = 42)

Three strong, vigorous hybrids which arose in the gardens and which were the only ones found, have been examined and appear sterile under both self- and open-pollination. There was 2.6% good pollen in the samples examined. Gene exchange between these species appears to be impossible, though it may be possible

between this F_1 and the other hexaploid species.

A. enysii × *A. scabrum* (2n = 35)

Natural hybrids of *A. enysii* × *A. scabrum* were observed in numbers at Porters Pass, inland Canterbury, at about 3000 ft. No seed was set on the plants observed during two flowering seasons. Pollen grains were stained and examined, no filled grains being found in 969 grains examined. It is concluded that these pentaploid hybrids are sterile.

The remaining combinations *A. enysii* × *A. kirkii* (2n = 35) and *A. kirkii* × *A. tenue* (2n = 49) have not arisen in experimental gardens, but have been artificially produced by J. B. Hair (pers. comm.), in whose experience they were sterile. Gene exchange between these combinations is therefore no more probable than in the recorded penta- and heptaploids.

Discussion

The results presented above and in an earlier paper (Connor, 1954) provide a basis for a biosystematic interpretation of the four species recognized as indigenous to New Zealand. Evidence available from morphology, ecology, genetics, and cytology, meets the basic requirements for an analysis of the evolutionary entities within the genus, as outlined by Clausen (1951). That the four species recognized from traditional studies on the morphology of the genus are distinct needs no repetition. The chromosome numbers of the species are listed in table 3.

Ecologically the species differ in geographical distribution and in range of habitats. *A. scabrum* is the most widespread of the species. It occurs throughout New Zealand, and occupies diverse habitats. It is found in the coastal and lowland regions along with *A. kirkii*, as well as in the damp situations favored by *A. enysii* and by *A. tenue*. *A. kirkii*, though predominantly a coastal and lowland species in North Island and northern parts of South Island, has been collected from low-tussock grassland on the Volcanic Plateau in North Island. It has not been collected in inland localities in tussock grasslands in South Island. *A. tenue*, though well distributed particularly in South Island, is largely restricted to damp or very shaded sites. *A. enysii* has the most restricted distribution of any of the four native species, as it appears to be confined to high altitudes in inland localities in Canterbury province. The known sites for this species are seepages, bogs, and sides of shaded streams. To date it

Table 3. *Summary of evidence for, and a biosystematic analysis in,* Agropyron

Species	Morphology	Ecology	Chromosome number	Genetic relationships	Biosystematic rank	Opportunity for natural hybridization
A. enysii	All Species Very Distinct (see Connor, 1954).	Damp sites high altitudes. Limited distribution in South Island only.	2n = 28	Sterile F_1 with *A. scabrum*. Sterile F_1 with *A. tenue*.	Coenospecies with a single ecospecies.	With *A. scabrum* and probably with *A. tenue*.
A. kirkii		Coastal and inland lowland areas in both Is. Limited distribution in one area of high altitude in North Island.	2n = 42	F_1 with *A. scabrum* of reduced fertility. F_2 sterile or unobtainable. First backcross generation has greater fertility than F_1.	Ecospecies (Coenospecies)	With *A. scabrum* and possibility of introgression. Very occasional with *A. tenue*.
A. scabrum		Coastal to high altitude, throughout N. Z. Sea-level to 5000 ft.	2n = 42	Sterile F_1 with *A. enysii* and *A. tenue*. See also under *A. kirkii*.	Ecospecies (Coenospecies)	With *A. kirkii* as above. With *A. enysii* and *A. tenue*.
A. tenue		Damp sites and shady slopes up to 3500 ft. More common in South Island.	2n = 56	Sterile F_1 with *A. scabrum*. Sterile F_1 with *A. enysii*.	Coenospecies with a single ecospecies.	With *A. scabrum* and probably with *A. enysii*. Very occasional with *A. kirkii*.

has not been seen growing together with *A. tenue* though the habitats are similar. The following four combinations of species have been found growing either in the same habitat or in immediately adjoining habitats separated by perhaps a few feet; *A. kirkii* and *A. scabrum, A. scabrum* and *A. tenue, A. enysii* and *A. scabrum, A. kirkii* and *A. tenue.*

Further evidence necessary for a biosystematic analysis of the New Zealand members of the genus comes from the studies on interspecific hybrids. The results detailed above show that gene exchange takes place between *A. kirkii* and *A. scabrum.* The vigorous F_1 hybrids examined were of reduced fertility. The only F_2 hybrids obtained were vigorous but proved sterile, no seed being set either under self- or open-pollination, and the pollen fertility being low. Backcrossing, or possibly interpollination among F_1 plants, led to a higher seed-set in F_1 plants, and plants raised from these F_1's showed an increase in fertility. Gene exchange can take place between these two species though to a limited extent. There is a possibility of introgressive hybridization occurring between these two species where their distribution is sympatric. The evidence indicates that *A. scabrum* and *A. kirkii* are two distinct ecospecies of a single coenospecies. They would qualify as *g*-ecospecies in the classification proposed by Valentine (1949). Though F_1 hybrids *A. enysii* × *A. scabrum* are found in nature, and *A. scabrum* × *A. tenue,* and *A. enysii* × *A. tenue* have

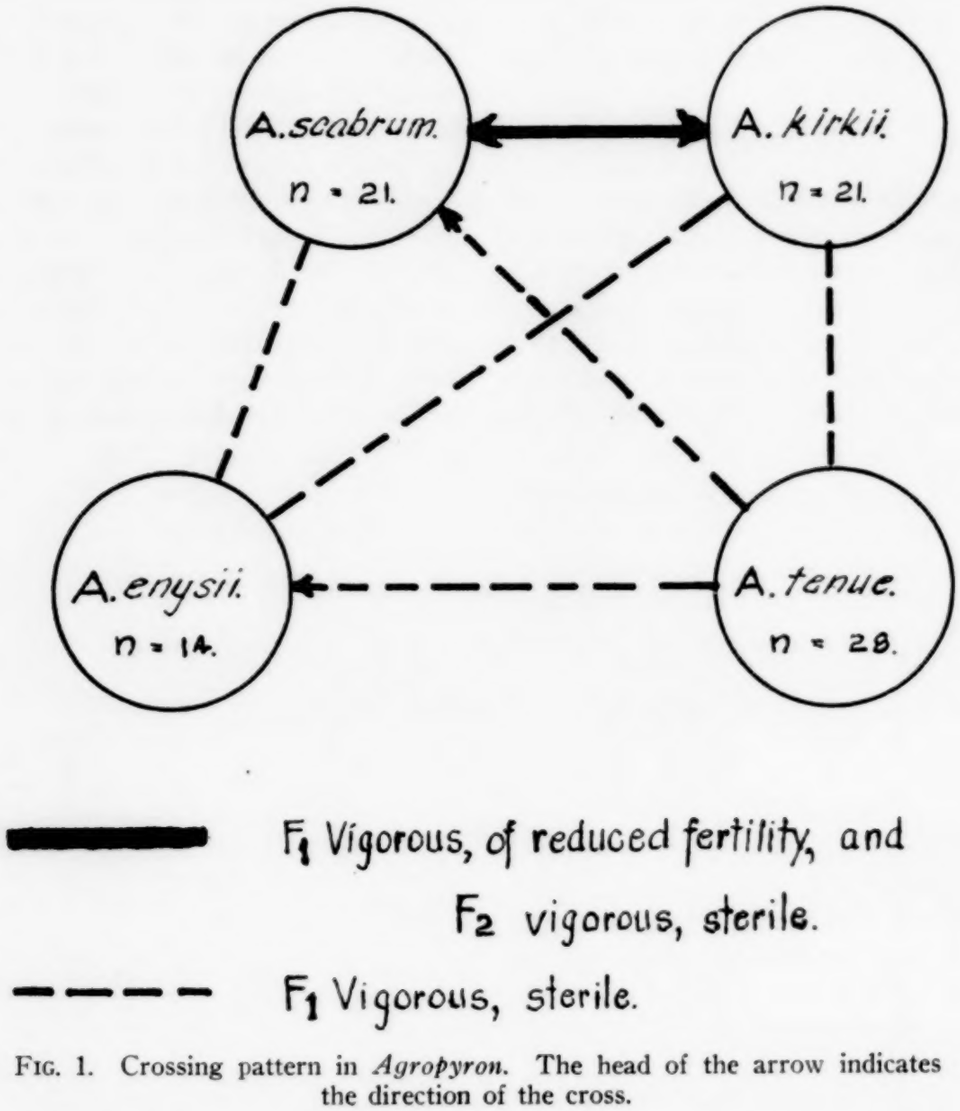

FIG. 1. Crossing pattern in *Agropyron.* The head of the arrow indicates the direction of the cross.

arisen in experimental gardens, no gene exchange appears possible as all the hybrids have so far proved sterile. These three sterile F_1 combinations have, in all instances, been growing where there is a considerable pollen supply of the parental species, but no effective pollination has taken place. In addition, F_1 hybrid plants of *A. enysii* × *A. tenue* ($2n = 42$) have been grown in plots where there was ample pollen from the 42-chromosome species, but again there has been no effective cross-pollination. *Agropyron enysii* and *A. tenue* are members each of separate coenospecies. Hybrids between these two coenospecies are possible but are sterile, as are hybrids between either of these coenospecies and the *A. kirkii–A. scabrum* coenospecies. The crossing pattern among the four species is shown in figure 1.

The opportunity for natural interspecific hybridization has been briefly and incompletely mentioned in a previous paper (Connor, 1954). In nature *A. enysii* crosses with *A. scabrum*. There is the possibility that *A. enysii* could cross with *A. tenue* should they co-exist in inland Canterbury. Though sympatric distribution of *A. enysii* and *A. tenue* has not been observed it probably does occur. As *A. enysii* and *A. kirkii* are allopatric, hybridization will not occur. *A. scabrum* and *A. tenue* are frequently of sympatric occurrence in South Island. Hybrids between these two species have not been seen in the field, though there is every possibility that they could arise. The distribution of *A. kirkii* and *A. tenue* is such that hybridization may occur in some few places, e.g., in a limited area on the Volcanic Plateau in North Island, though no hybrids have been found. Hybrids between *A. kirkii* and *A. scabrum* are likely where the two species have common distribution. There is also the possibility that introgression may take place between these two. An examination of herbarium specimens suggests that there has been natural hybridization between these taxa, followed by backcrossing. The hybrids reported on above indicate that it is between these species that this phenomenon is likely to occur.

The results from these studies may now be related to the taxonomic treatment of the genus in New Zealand. Clausen *et al.* (1945) state that the biosystematic units which are investigated by experimental methods are ". . . usually found to correspond fairly closely to taxonomic units based on careful morphologic and distributional studies." The conclusions reached on the biosystematic status of the four native species here discussed are in agreement with those reached from a study of the morphology of the plants. All four species stand as "good" species.

The evidence from which the biosystematic status of the New Zealand members of the genus *Agropyron* may be determined is summarized in table 3.

Summary

Data on interspecific hybrids in *Agropyron* are reported, and are used together with other available evidence to define the biosystematic status of the species. The results indicate that the indigenous species belong to one comparium and consist of three distinct coenospecies, *A. enysii* coenospecies, *A. tenue* coenospecies, and *A. scabrum–A. kirkii* coenospecies with two ecospecies. The taxonomic treatment previously reported is in agreement with the results of these studies.

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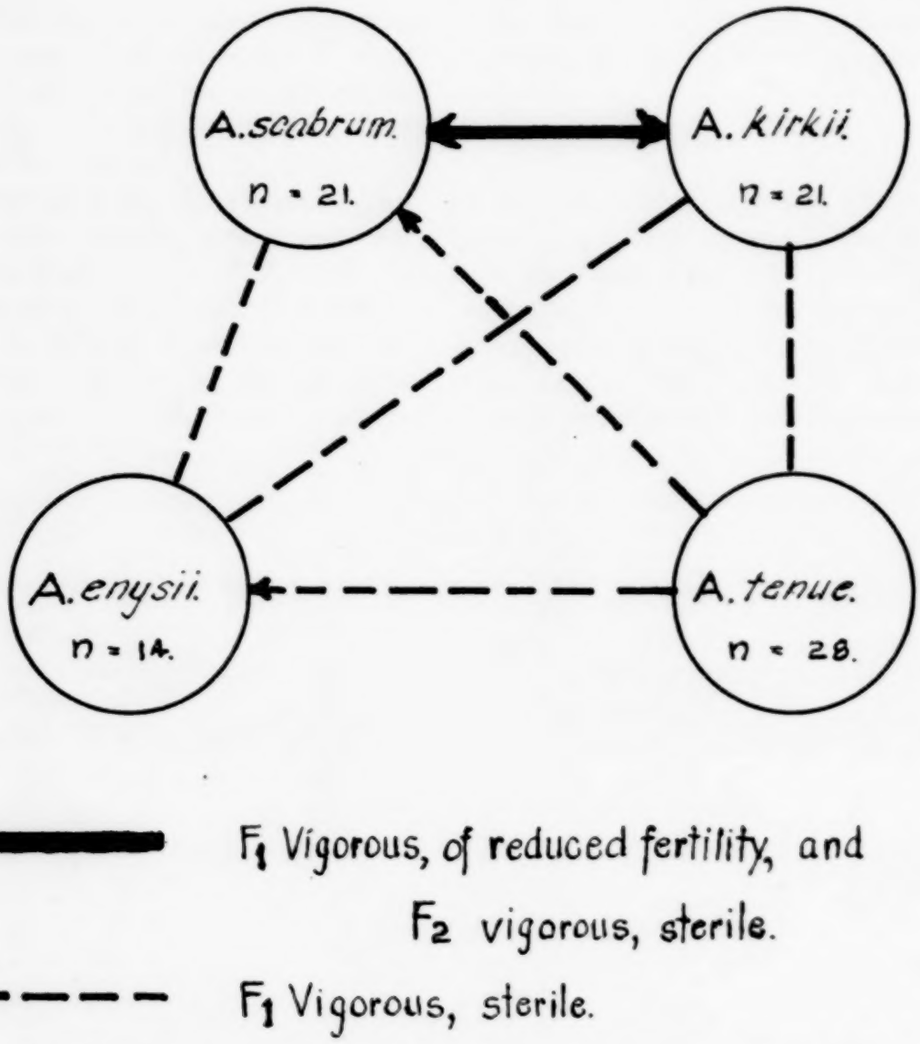

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Island, while A. A. Sutherland, Botany Division drew figure 1.

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A GENE MUTATION WHICH CHANGES A BEHAVIOR PATTERN [1]

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INTRODUCTION

It is tempting to assume that behavior patterns have evolved in the same manner as other features of animal populations, by the selection of inheritable differences. This idea is supported by those comparisons which have been made between the behavior patterns of closely related animals, one of the best examples of which is the review by Spieth (1952) of the courtship behavior of 101 species and subspecies of the genus *Drosophila*. Spieth compares the degree of morphological with that of behavioral divergence and he finds that the two roughly agree so that mating behavior confirms the validity of the existing classification as presented by Patterson and Wheeler (1949).

Yet it is impossible to be certain, however strong the implication about the evolution of behavior, unless one can demonstrate the existence of 'genetic' variations in behavior upon which selection could act. It is true that some gene mutations are known which affect behavior in some way. Thus one mutation in *Panaxia dominula* has influenced its mating 'preferences' (Sheppard, 1952), the ebony mutant in *Drosophila melanogaster* mates more successfully in the dark than in the light (Rendel, 1951), and there is a mutation which affects tameness in rats (Keeler and King, 1942). But the differences in the courtship behavior of the *Drosophila* species studied by Spieth and also by Milani (1951a, b) are of a more specific nature. Courtship is a complex behavior pattern and the variations often occur only in the relative frequency of the elements concerned, some elements occurring more often in one species than another. Sometimes elements are omitted or new ones are added and sometimes they may change in form, but the most common difference between the most closely related species concerns this difference in frequency. The investigations reviewed by Moynihan (1953, 1955) on the differences between closely related gulls and the descriptions given by Clark, Aronson and Gordon (1954) on the differences between the platyfish (*Xiphophorus* (*Platypoecilus*) *maculatus*), and the swordtail (*Xiphophorus helleri*), suggest changes of the same nature, and yet no single gene mutation is known which alters a behavior pattern in this way. However since considerable detailed investigation is necessary to reveal differences in frequency or patterning, this is not so very surprising. Indeed some of the mutations listed above might well have such effects; they simply have not been investigated. It seemed worth while therefore to examine more closely one example of a gene mutation affecting behavior and to ask two questions, (1) how does it bring about its effect? (this is of considerable genetical interest), (2) what part might it play in evolution? (this is of considerable evolutionary interest).

MATERIAL USED

The mutant which I chose to investigate was the yellow mutant of *Drosophila melanogaster*. This is a recessive sex-linked gene located at one end of the X chromosome, the adults being clearly distinguished from wild type because the body is yellow, instead of the normal gray,

[1] This work is part of that submitted for the degree of Doctor of Philosophy, in the University of Oxford. March 1955.

and because the transverse bands on the body are dark brown instead of black. The larvae are also recognizable because their setae and mouth parts are yellow-brown instead of dark brown; in fact yellow insects seem to be generally deficient in melanin in spite of the fact that they contain more of the enzyme tyrosinase (responsible for the melanin reaction) than do wild type insects (Graubard, 1933). The mutant arises frequently, but although yellow insects are very successful as laboratory stocks they are not frequent in nature, probably because of their lack of mating success. This was first noted in 1915 by Sturtevant, who showed that yellow males are usually unsuccessful in competition with wild males for females. It seemed possible that this could be because their behavior had changed in some way so as to make them less stimulating.

I demonstrated the nature of the effect by comparing the success of single pair matings of wild and yellow males with wild females. My two stocks (wild and yellow) had previously been made genetically similar by intercrossing for seven generations, and in this, as in all other experiments and observations quoted in this paper, the flies were four or five days old, males and females having been kept for this time after emergence in separate vials. Figure 1 is a cumulative graph in which the percentage of successful males of each type is plotted against time. Clearly although the yellow males do eventually mate they take much longer to do so. Moreover although yellow males take slightly longer before they begin to court (an average of 9.6 minutes as compared with 4.9 minutes), it is also true that they have to court for longer before they can mate. Yellow males take on the average 10.5 minutes from the beginning of courtship to copulation, wild males only 6.0 minutes. These figures were all obtained from one series of experiments because each pair was watched and the times of introduction, courtship commencement,

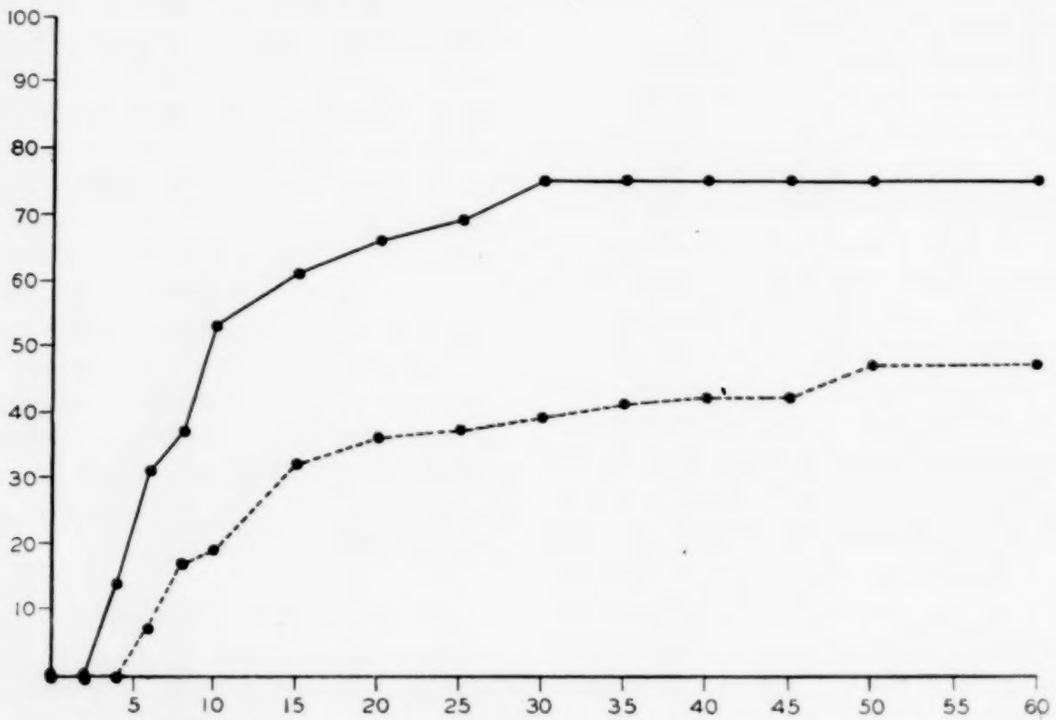

FIG. 1. Mating success compared for wild and yellow males with wild females in paired matings. The wild and yellow stocks had been previously crossed for 7 generations. Average time from introduction to copulation: wild male × wild female: 9.1 minutes; yellow male × wild female: 18.9 minutes. Abscissa: time in minutes from introduction. Ordinate: % successful males. Dashed line: yellow male × wild female. Solid line: wild male × wild female.

copulation, were noted for each. All the differences quoted above are significant at the 1% level.

It is clear, therefore, that yellow males must be less stimulating in some way than wild ones. This could be because they look or smell different or it could be because their courtship has changed. Because the courtship is so prominent a feature of *Drosophila* pre-copulatory behavior it is likely that it plays a dominant role in stimulating the females and it therefore seemed worth while investigating this first. One point is clear; yellow males fall very little short in the persistency of their courtship. I watched 19 wild males and 19 yellow males separately courting wild females and recorded their activity every 1½ seconds for a period of 2½ minutes each. The wild males courted for an average of 92% of the time, the yellow ones for 83%. This difference is significant at the 5% level but it seems too small to account entirely for the reduced success of the yellows. I therefore looked for a possible reduction in the stimulating quality of the courtship itself.

Description of Courtship

The courtship of *Drosophila melanogaster* is too well known to need a detailed description. When a male is put into an observation cell with a virgin female (preferably after both have been isolated for about 3–5 days from eclosion), he will very soon approach her and tap her with his fore-legs. After tapping, he may not immediately begin to court; he may clean or walk away. If so, then he will sooner or later tap again and eventually he will follow this by courtship. After starting courtship, however, he rarely taps a second time except perhaps after a long break.

At the beginning of courtship the male stands facing the female, usually in the position from which he has approached, whether it be at the side of, in front of or behind her. (Spieth says that he always circles to the rear immediately but I do not agree with this, and Sturtevant also observed that he would stand in any position relative to the female.) If the female moves off, the male will follow, and this of course brings him behind her, but often the female stands still and then he does not at first change his position. I have called this part of the courtship *orientation,* irrespective of whether the male is following or standing.

The wing display usually follows after a few seconds of orientation. One wing is brought out until it is at right angles to the male's body, then it is vibrated rapidly up and down for a few seconds before being returned to rest. This may be repeated several times at short intervals during which time the male may be following the female or standing facing her. Eventually if he is not already behind her, he will move into this position, although he may first circle right round her head, and sometimes, even from an original position behind her, he may move first to one side and then to the other before finally staying behind. It is noticeable, during these maneuvers, that he always vibrates the wing nearest to the head of the female, usually changing wings as he passes her head or her tail.

Finally, once the male is behind the female, he licks her genitalia with his proboscis and attempts to copulate. This involves curling his abdomen under his body and jerking it towards the vaginal plates of the female meanwhile grasping her abdomen with his forelegs. He will not succeed however in inserting his phallus unless the female cooperates and spreads her vaginal plates.

The wing display I have called *vibration,* and licking and attempted copulation are collectively termed *licking.* I have not separated these two because it appears that licking is always followed by attempted copulation unless the female is able to prevent it (by kicking or twisting her abdomen or running away).

During vibration the male is always orientating, and during licking he is both orientating and vibrating; these three ele-

ments are therefore not distinct but are superimposed upon one another. Figure 2 gives a representation of the patterning of these elements in time. It shows records of the courtships of four males, the activity of each being noted every 1½ seconds. It will be seen that a given male changes rather regularly from one activity to another, but that males vary in the proportions and in the bout lengths of the different elements.

The Stimulating Elements of the Courtship

To return to the problem of the yellow males, it seemed of interest to discover the relative stimulating value of these elements, and since vibration is the most prominent and the most peculiar feature of the courtship, I investigated this first. It is easy to eliminate vibration alone, for males whose wings have been removed (about 2 days previously under ether) will court just as persistently as normal ones. Detailed observations on ten wingless males showed that they courted for 89% of the time as compared with 86% for the winged controls. Moreover they court quite normally. One can see them 'vibrating' their wing stumps, and wingless males licked an average of 5.8 times in a 2½ minute observation period as compared with the 6.1 times of winged males. These differences are not statistically significant.

Figure 3 gives the results of some mass mating experiments which I performed with 5 males (all of one type) and 10 females (all of one type) together in vials for two hours, the females being dissected afterwards to discover which had been fertilized. It can be seen that wingless males are very much less successful than normal ones (32% success in 2 hours as compared with 70% : $P < 0.01$). They are equally unsuccessful if the experiments are done in the dark; indeed light does not appear to make very much difference to the success of either type of male, the figures in the dark being 31% for wingless and 75% for winged males. This agrees with the findings of Spieth and Hsu (1950) that visual stimuli are not important in the courtship of *Dro-*

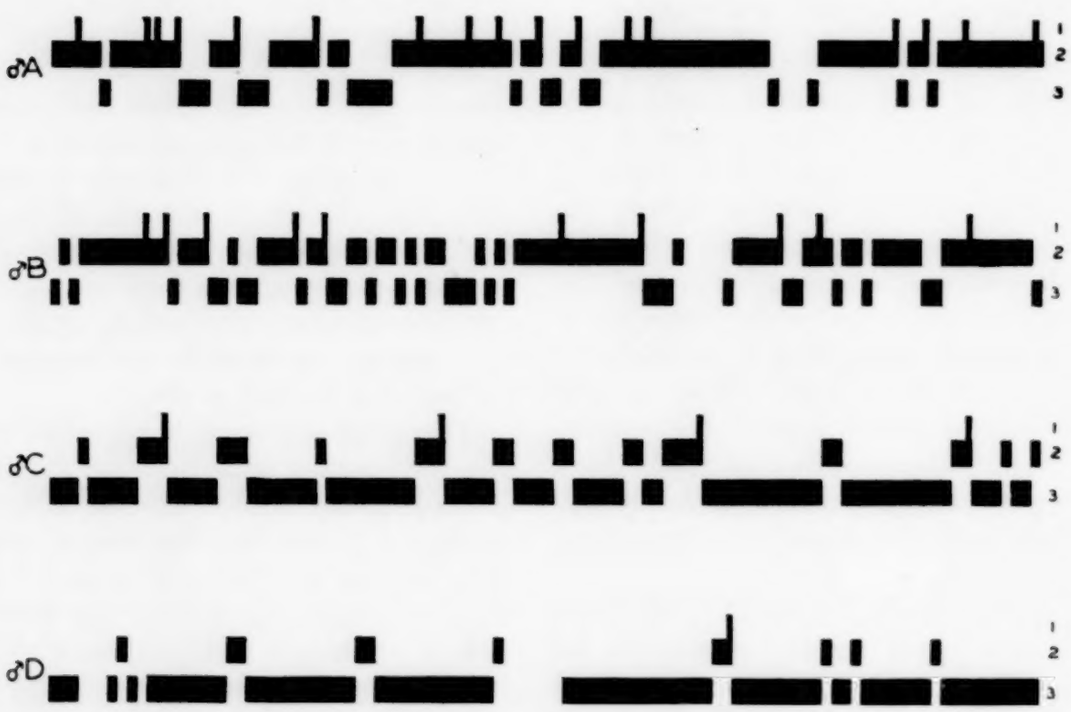

Fig. 2. Diagrammatic representation of the courtship patterns of four wild *D. melanogaster* males, each with a virgin wild type female. 1. licking; 2. vibration; 3. orientation.

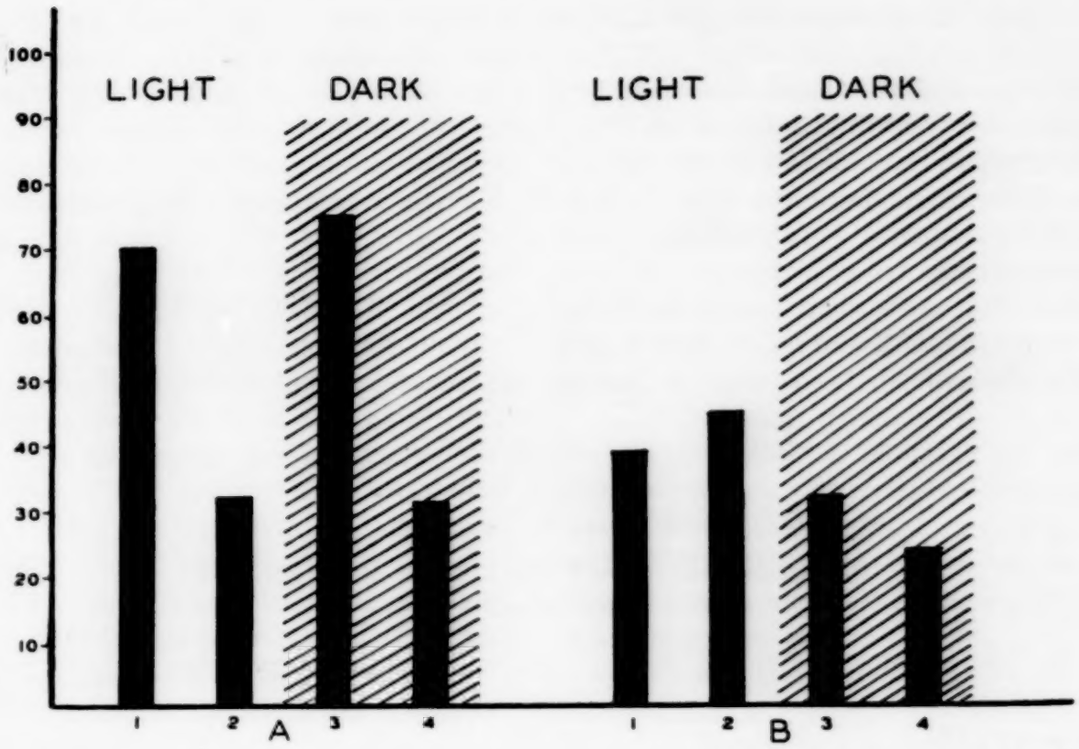

FIG. 3. Results of experiments performed simultaneously with winged and wingless males, normal and antennaless females. 5 males and 10 females per vial. 9 vials in each category. Abscissa: A. normal ♀♀; 1. winged ♂♂, 2. wingless ♂♂, 3. winged ♂♂, 4. wingless ♂♂. B. antennaless ♀♀. 1, 2, 3, and 4, as for A. Ordinate: % females fertilized.

sophila melanogaster. It also shows that the stimuli provided by wing vibration are not visual, for both the undiminished success of winged insects in the dark and my own observations confirm that courtship is quite normal in the dark.

The other experiments shown in figure 3 were run simultaneously with those just quoted, but antennaless rather than normal females were used (like the wings, the antennae were removed from anesthetized insects 2 days before the experiments and to counteract possible effects of ether, all normal flies used in these experiments were also anesthetized at the time of the operations). It is clear that antennaless females do not discriminate between winged and wingless flies, either in the light or in the dark (such differences as appear in the figures are not significant). Moreover it seems that the success of both types of male with these females is reduced by the same amount as that of wingless males with normal females. In other words it is likely that the same stimulus is lacking in both cases, i.e. the stimulus produced as a result of vibration, and that this is perceived by the antennae of the female. These findings explain the role of the antennae in the mating behavior of females of this species demonstrated by Mayr (1950) and Begg and Packman (1951). The fact that the two latter authors demonstrated that removal of the male's antennae also reduced mating success suggests that olfactory stimuli are important also to them.

The degree of the drop in success is evidence that vibration is a very important stimulating element in the courtship and that orientation and licking (which are present in wingless males) are relatively less important. Licking probably is of some stimulus value but these experiments suggest that it plays a relatively minor role, thus agreeing with Spieth's suggestion (1952) that licking is more important in the species of the sub-genus *Drosophila*, where it tends to be more prolonged, than in the sub-genus

Sophophora of which *melanogaster* is a member.

Thus it is apparent from this investigation that any courtship deficient in vibration is likely to be considerably less stimulating than one containing a high proportion of this element. Now an examination of the courtship records shown in Figure 2 shows that individual males do differ both in the amount, and in the bout length of this element, males A and B giving higher values of each than C and D. The males pictured here were all wild males, but males like C and D are relatively rare, while A and B may be described as typical. It seemed worth while to see if this was also true of yellow males.

The Difference Between the Courtships of Yellow and Wild Males

In these experiments I wished to avoid as far as possible the effect of genes other

Table 1. *Analysis of courtship of wild and yellow males with wild females*
Experiments with 'brother' males

All insects *D. melanogaster*

100 records made for each pair at intervals of 1½ seconds

	Wild males × wild females						Yellow males × wild females					
	T.R.T.	O%	V%	L%	Av. V+L	Av. O	T.R.T.	O%	V%	L%	Av. V+L	Av. O
Day 1	100	78	19	3	2.7	6.5	95	76	20	4	2.6	6.7
	98	73	17	10	3.8	4.4	98	94	5	1	2.5	25.8
	68	62	37	1	4.3	4.2	95	82	12	6	2.8	5.8
	81	75	19	6	3.5	5.6	86	90	7	3	2.6	11.0
	88	80	18	2	3.1	8.5	83	77	18	5	3.3	4.8
	83	72	27	1	3.4	5.1	70	71	28	1	2.7	6.6
Day 2	96	69	24	7	4.6	5.7	30	73	14	13	3.3	5.8
	99	81	15	4	3.0	6.3	97	78	16	6	3.3	5.6
	82	82	17	1	3.0	10.8	93	80	17	3	2.9	7.3
	100	60	32	8	4.4	3.1	100	65	25	10	3.3	3.2
Day 3	86	87	12	1	2.8	7.6	85	86	13	1	2.8	13.8
	97	72	22	6	3.6	5.2	51	67	27	6	2.8	4.0
Day 4	100	74	20	6	3.6	5.6	85	78	16	6	2.8	6.2
	88	73	24	3	3.4	6.7	89	72	22	6	2.9	4.1
Day 5	100	51	44	15	6.2	2.4	98	73	24	3	3.1	4.5
	94	74	17	9	3.6	4.5	92	74	16	10	4.1	4.8
	100	61	24	15	6.9	2.9	92	75	14	11	2.7	4.1
Day 6	95	68	23	9	3.3	4.7	75	79	16	5	2.9	4.9
	85	74	20	6	4.9	5.0	68	69	24	7	3.2	3.0
Av. for 19 pairs	92	72	22	6	3.9	5.5	83	77	18	6	2.9	6.9

T.R.T. = no. of records out of 100 in which courtship activities were shown.
O% = percentage orientation in the courtship records.
V% = percentage vibration in the courtship records.
L% = percentage licking in the courtship records.
Av. V+L = average bout length of vibration plus licking expressed in units of 1½ secs.
Av. O = average bout length of orientation (calculated in the same way as Av. V+L).

Results of analysis of variance: Significance of differences between male types

T.R.T., $P < 0.05$; O%, $P < 0.05$; V%, $P < 0.05$; L% not significant; Av. V+L, $P < 0.01$; Av. O, not significant.

than yellow. I therefore crossed my two stocks together (yellow females × wild males) and then mated the daughter heterozygous females to their brother yellow males. The sons of these females were wild and yellow in equal numbers, and it may be assumed that any other genes for which their parents were heterozygous (i.e. 'stock' differences) should assort independently of the wild and yellow genes, unless very closely linked. I did not in any case expect there to be many such differences as it was only a few generations since I had crossed my two stocks together for 7 generations.

Table 1 shows the results of comparisons of the courtships of wild and yellow males. The insects were recorded for the first 2½ minutes of their courtship with wild females, each couple being alone in an observation cell and the male's behavior being noted every 1½ seconds. Wild and yellow males were recorded in turn and all those recorded on a given day were of the same age, i.e. either four or five days from emergence. From the records I could count the total number of courtship activities (disregarding periods when the male was not courting) and I could then calculate the percentage of that courtship which consisted of each of the three elements, orientation, vibration and licking. By this means I could assess the stimulating quality of the courtship itself, irrespective of the effect of breaks. It can be seen that yellow males show a slightly lower percentage of vibration (the difference is significant at the 5% level) and this is compensated, not by licking, which is probably also stimulating, but by orientation (the difference is again significant at 5%). An even greater difference is found if one calculates the average bout length of vibration. Reference to Fig. 2 will show that this really means the average bout length of vibration plus licking, since licking is momentarily superimposed during a vibration bout. The bouts shown by wild males are considerably longer than those shown by yellow ones and this difference is significant at 1% level. The orientation bouts also appear to be longer for yellow males although this is not significant on these figures. However I have presented evidence elsewhere (Bastock and Manning 1955) that vibration bout length is negatively correlated with orientation bout length, so that it appears that these mutants tend to vibrate for shorter periods and at longer intervals than do wild males. This could be very important if perhaps a greater concentration of the stimulating elements is significant to females. Bursts of scent or sound might need to be of a certain duration before they are of any stimulus value, and their effect may tend to die down between bursts.

The Nature of the Effect of the Mutation

Having established a difference in behavior which could well account for the reduced success of yellow males, it is not legitimate to cease the investigation at this point. In the first place, it is possible that changed behavior, changed color, and perhaps changed scent, are all separate effects of this mutation and perhaps not only one, but several or all of them may diminish mating success. But even more important is the possibility that the behavioral change is not a primary effect of the mutation at all. A male's courtship behavior is never an entirely automatic process; it is determined, at least in part, by the stimuli received from the courted object. It is therefore quite possible that a female, reacting against the changed appearance or scent of a yellow male, may either fail to give attractive stimuli or give instead repelling stimuli. Thus the male's deficient courtship behavior may be explained simply as a different reaction to different stimuli, and there may be no fundamental difference between the two males in this respect at all.

This second possibility can be investigated in various ways.

(1) In the first place it is possible to consider the behavior of the females dur-

TABLE 2. *Number of records of repelling movements made by melanogaster and simulans females with wild and yellow melanogaster males*

Out of a total of 100 records per pair

Experiments with 'brother' males

A.

Wild male × wild *melanogaster* female							Yellow male × wild *melanogaster* female						
Pair	J	K	FL	TW	EX	Total	Pair	J	K	FL	TW	EX	Total
(1)	1	3	1	1	0	6	(2)	0	10	0	0	0	10
(3)	3	0	7	5	0	15	(4)	2	3	5	0	5	15
(5)	6	0	0	3	4	13	(6)	6	1	4	3	0	14
(7)	3	4	4	0	0	11	(8)	1	0	6	1	0	8
(9)	2	5	2	3	0	12	(10)	2	1	0	1	1	5
Total	15	12	14	12	4	57	Total	14	15	15	5	6	52

B.

Wild male × *simulans* female							Yellow male × *simulans* female						
Pair	J	K	FL	TW	EX	Total	Pair	J	K	FL	TW	EX	Total
(11)	0	8	10	20	0	38	(12)	2	7	0	7	0	16
(13)	2	7	1	19	0	29	(14)	1	13	0	5	1	20
(15)	1	9	0	6	1	17	(16)	0	13	3	0	0	16
(17)	3	17	5	1	0	26	(18)	0	18	0	1	0	19
(19)	2	9	1	3	0	15	(20)	0	15	6	6	1	28
Total	8	50	17	49	1	125	Total	3	66	9	19	2	99

J = jumping.
K = kicking.
FL = flicking wings.
TW = twisting abdomen.
EX = extruding genitalia.

ing the courtship. Although females give no obvious acceptance or 'attractive' stimuli, an unwilling female gives clearly recognizable repelling movements. She may kick at the male, she may twist her abdomen so that he cannot copulate, she may flick her wings (a movement similar to that made by males courted by other males), she may extrude her genitalia (a movement made only rarely by virgins but frequently by fertilized females) and finally she may jump or fly suddenly away. It seems legitimate to suppose that if females are for some reason reacting against yellow males, then they will show more of these movements when courted by them. Table 2A shows that this is not so. Here the repelling movements made during 2½ minutes of courtship are tabulated for 10 females, five of them courted by wild males and five by yellow ones. In fact slightly fewer of these movements are made to yellow males (although the difference is not significant), and it is also clear that there is no great difference in the proportion of the different types of movement made to the two males.

(2) It is also possible to measure how far a female tends to stand still or run around the cell while being courted by the two types of male. This is important because I have shown that significantly more vibration and less orientation are directed to a standing than to a moving female (see fig. 4). (The figures are 49% orientation and 41% vibration given to a standing female; 68% orientation and 24% vibration given to a moving one;

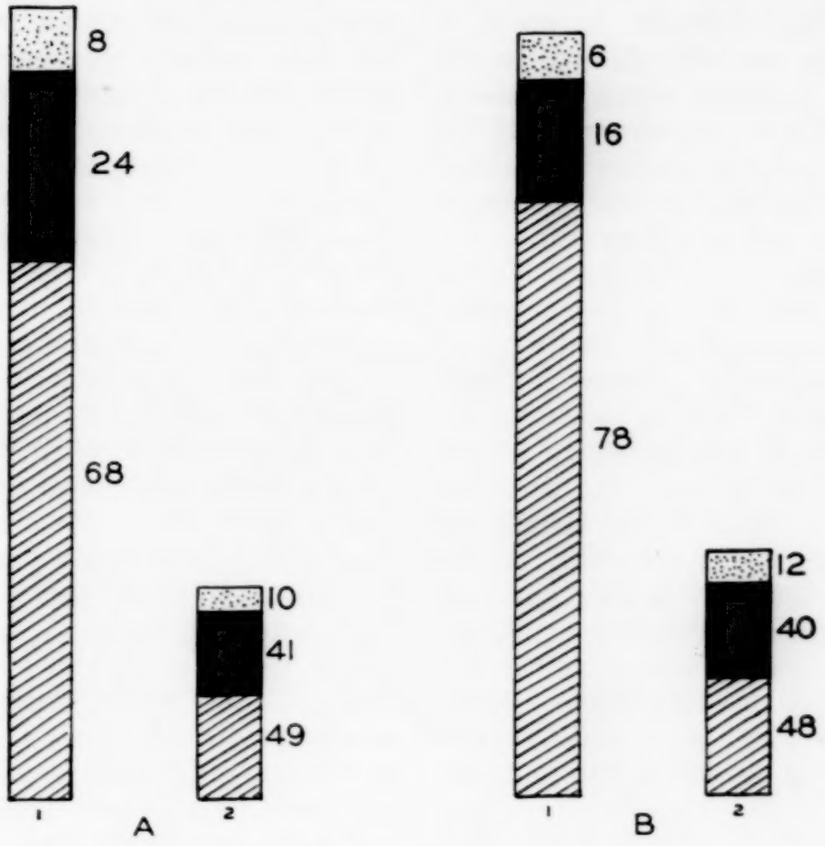

FIG. 4. Diagram illustrating the proportion of time spent running or standing by females courted by wild or yellow males (indicated by the height of columns). The composition of the courtship under these two conditions of females is shown by shading of the columns. The figures are calculated from 2½ minute records of 23 pairs of each type. A. wild type males; 1. ♀ running for 79% of the courtship, 2. ♀ standing for 21% of courtship. B. yellow males; 1. ♀ running for 76% of the courtship, 2. ♀ standing for 24% of the courtship. Key: hatchured areas: % orientation; solid areas: % vibration; stippled areas: % licking.

differences significant at the 1% level in each case.) This may be because the male cannot keep so close to the female and is therefore less stimulated. Whatever the reason, if it could be shown that females courted by yellow males tend to run for a greater proportion of the time, this might well explain the difference in courtship pattern. However, once again, this is not the case. 23 females courted by yellow males, each for 2½ minutes ran for 76% of the time, while a similar number of females courted by wild males ran for 79% of the time. There is no significant difference between these figures but in any case its direction is that which would favor yellow males giving more, not less, vibration in their courtship.

(3) Another line of approach to this problem is to consider the courtship given by these two types of male to females which reject them both (the assumption being that they would reject both equally). Ideally one should compare their courtship to a standard stimulus object, such as a model, but I have not succeeded in inducing *melanogaster* males to court models. (Milani (1951b) induced such behavior only in those species of the *obscura* group whose mating success decreased considerably in the dark, i.e. only in those which gave great 'weight' to visual stimuli. Since *melanogaster* males respond mainly to olfactory and tactile stimuli, my failure is not surprising.) *Melanogaster* males will court dead fe-

males, but their behavior to these is highly variable, probably due to the degree of decomposition, which is difficult to control. They do however court the females of the sibling species *Drosophila simulans* very persistently and apparently normally, although they are rarely accepted by them. So I compared the behavior of the two males to these females, in experiments performed exactly as for *melanogaster* females, using 'brother' males as before. Table 3 shows the results and gives an exactly similar picture of the distinction between the courtship of the two male types, except for the fact that, because of higher variability, in this case only the difference in average vibration bout length is statistically significant. This however is significant at the 1% level. Confirmation that *simulans* females do tend to reject both males equally comes from counts of inhibitory stimuli, detailed in Table 2B. Once again slightly more were given to wild than to yellow males.

(4) Finally it seemed worth while comparing the relative success of wild and yellow males in the dark and with antennaless females. If the females react against yellow males because of changed scent or changed appearance then one would expect the elimination of these stimuli to reduce the difference in success between the two males. Figures 5 and 6 show the results of pair mating experiments designed to test this. In neither case is the difference reduced, although in the case of antennaless females the success of both types of male is considerably lowered. Clearly then neither changed appearance nor changed scent is a primary cause of the yellow male's failure. It cannot be argued that these factors play no role, only that in the absence of either type of stimulus, differences between the males still remain. It is probable that these are behavioral differences.

The above arguments therefore strongly suggest that a less stimulating courtship is a characteristic of the yellow male and does not seem to be attributable to a difference in the behavior of the courted object to the two types of male. It is necessary at this point to say something about the situation when yellow females are used instead of wild ones. These females accept yellow males almost as readily as wild ones, and what difference does exist can be attributed mainly to the fact that yellow males do not begin courting quite so soon. There is very little difference in the length of actual courtship required before copulation, and this is undoubtedly puzzling if we believe that yellow males are less stimulating. It could be explained if the yellow females were much more highly receptive than the wild ones, i.e. they would not then require so much stimulation. But although yellow females taken from yellow stock bottles often *are* more receptive than wild type ones, I have evidence which I give in detail later (see page 436) that this is due to genes other than yellow, and must be selected for in yellow stocks. I have not yet solved this problem as I have not yet sufficient de-

TABLE 3. *Analysis of the courtship of wild and yellow* melanogaster *males with* simulans *females.*
Experiments as in table 1 using 33 males of each type. Only the average results are shown.
Experiments with 'brother males'

	Wild males × *simulans* females						Yellow males × *simulans* females					
	T.R.T.	O%	V%	L%	Av. V+L	Av. O	T.R.T.	O%	V%	L%	Av. V+L	Av. O
Av. for 33 males	86	61	29	10	5.2	4.1	90	64	27	9	4.2	4.3

Results of analysis of variance: Significance of differences between male types

Av. V+L, $P < 0.01$. All others not significant.

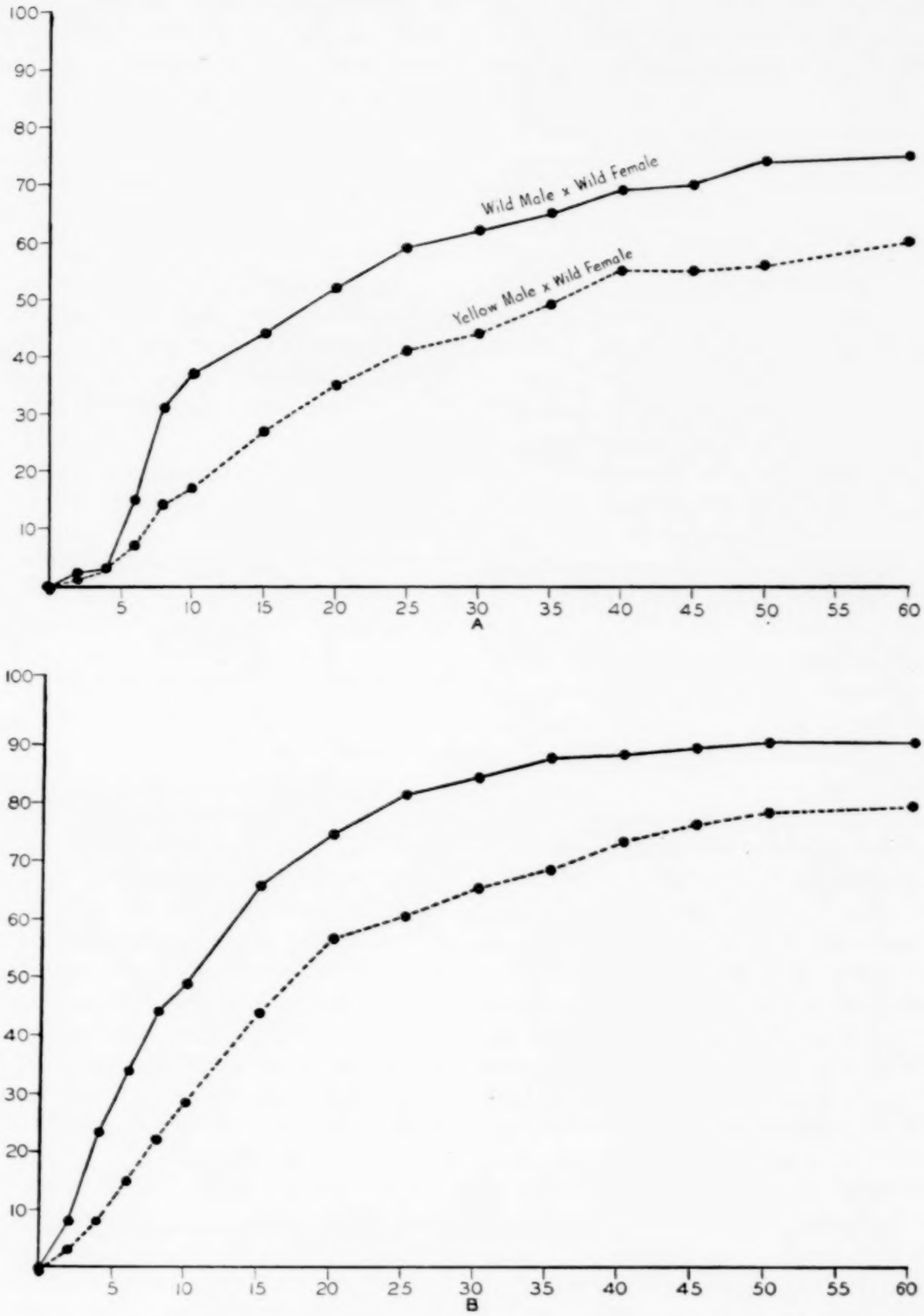

FIG. 5. A. Mating success compared for wild and yellow males with wild females in DARK in pair matings. Average time from introduction to copulation in DARK; wild male × wild female: 16.0 minutes; yellow male × wild female: 20.9 minutes. B. Controls tested simultaneously in the LIGHT. Average time from introduction to copulation in LIGHT: wild male × wild female: 11.4 minutes; yellow male × wild female: 17.6 minutes. The wild and yellow stocks had been previously intercrossed for 7 generations. Abscissa: time in minutes from introduction. Ordinate: % of successful males. Dashed line: yellow male × wild female. Solid line: wild male × wild female.

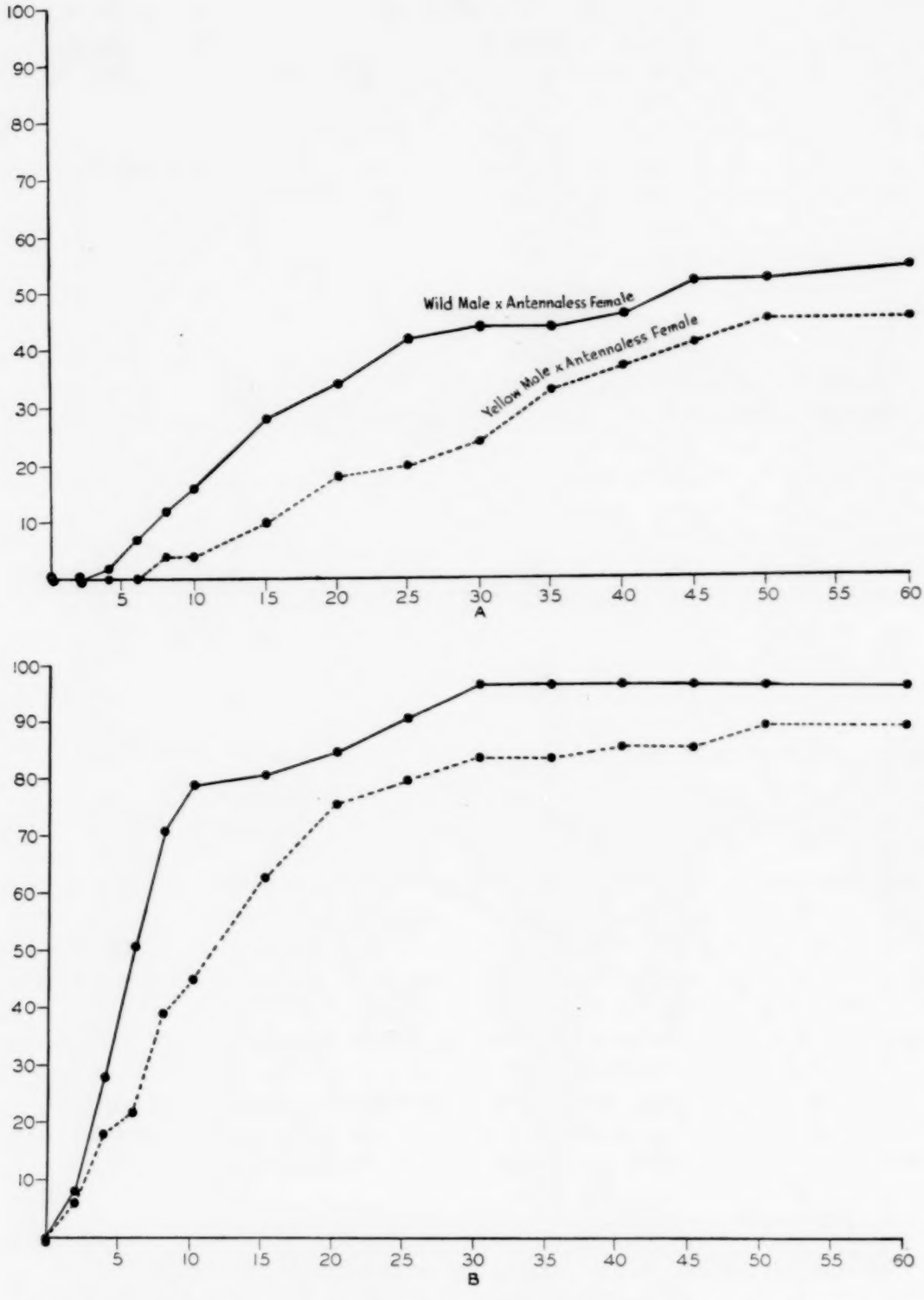

FIG. 6. A. Mating success compared for wild and yellow males with wild ANTENNALESS females in pair matings. Average time from introduction to copulation: wild male × antennaless female: 19.3 minutes; yellow male × antennaless female: 27.3 minutes. Dashed line: yellow male and antennaless female. Solid line: wild male and antennaless female. B. Controls tested simultaneously with NORMAL females. The wild and yellow stocks had been previously intercrossed for 7 generations. Average time from introduction to copulation: wild male × normal female: 8.4 minutes; yellow male and normal female: 12.5 minutes. Dashed line: yellow male and normal female. Solid line: wild male and normal female. Abscissa: time in minutes from introduction. Ordinate: % successful males.

tailed analyses of courtships directed to these females. Those I have, however, suggest that yellow females may stand still more when courted, and since there is less difference between the two courtships when directed to a standing female (see fig. 4), this could reduce the distinction between the two types of male. Thus this behavioral difference is to some extent dependent upon the behavior of the courted object and it will be greatest when that object is very active. But there is still a fundamental difference between wild and yellow males, for they behave differently to the same object, but more differently to some objects than to others.

The way in which this gene mutation brings about this difference is of course of great interest. There are certain clues in the results which I have already quoted. Thus besides changing their pattern of courtship, yellow males also take slightly longer to begin courting and court slightly less persistently (see p. 423). Also the results just quoted showing that their success is still reduced even with antennaless females means that besides being deficient in the major stimulus of wing vibration (perceived by the female's antennae), they are also deficient in other minor stimuli which serve to rouse the female when vibration is absent. All this suggests that the behavior of the yellow male is generally less vigorous than that of the wild one, and that the reduction of vibration is only one aspect of this.

This idea is amply confirmed by other observational evidence which is difficult to quantify. For instance, when following, yellow males rarely keep up so well with the females, and in wing vibration too, the whole activity seems less efficient, often the wing is not extended to its full 90° angle with the body, and it looks as if the amplitude of the vibrations may be reduced. The licking too seems a weaker movement and often, if the female is running fast, or if she kicks, a yellow male will fail to bring his proboscis in contact with her genitalia. These observations are in line with those of Sturtevant (1915) for *melanogaster* and Rendel (1945) for *subobscura* who both suggested that yellows were more lethargic in their courtship.

The changed pattern of courtship is therefore clearly associated with reduced general courtship vigor. Is it possible to ascribe both to the same cause? In another paper (Bastock and Manning, 1955), we have investigated the organization of the behavior pattern of courtship. I have described earlier that the three elements, orientation, vibration and licking, are superimposed upon one another, and we examined the possibility that each element appears at a different threshold of a common motivation. Figure 7 is a diagrammatic representation of our hypothesis. Three assumptions are made: (1) that the motivation fluctuates regularly, (2) that the elements occur at different thresholds of this motivation, and (3) that during courtship the motivation remains for most of the time within the limits set by these thresholds. If the first two assumptions are accepted, the third is justified by the fact that courtship is mainly continuous with few breaks.

Predictions can be made from this hypothesis which can be tested. We made, and verified three such predictions. (1) Vibration bout length will be inversely correlated with orientation bout length, i.e. if the motivation is for long periods above the threshold for vibration, it will tend to be below it for short periods and v.v. We obtained an inverse correlation from records of a large number of males ($r = -0.61$, $P < 0.01$). (2) There will be a positive correlation between vibration bout length and licking, i.e. if the motivation is for a longer period above the threshold for vibration then it will rise for longer above that for licking. Here $r = 0.64$, $P < 0.01$. (3) It will be possible to correlate high vibration bout length, taken as a measure of high average motivation, with some independent assessment of this. For an independent measure we took high 'total response time' over a long period, i.e. the amount of time

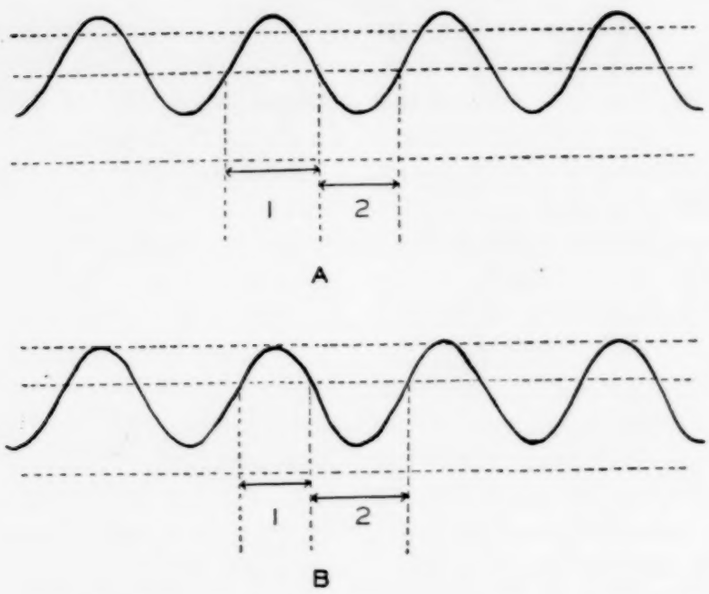

FIG. 7. Diagram of the suggested way in which a change in average sexual motivation affects the courtship pattern. Motivation is assumed to fluctuate regularly in relation to the three thresholds at which the elements appear. A. High average motivation produces courtship typical of wild type males. B. Low average motivation produces courtship pattern typical of yellow males. 1. Bout length of vibration and licking. 2. Bout length of orientation.

which a male would spend courting a non-responsive object (e.g. a *simulans* female) before he became 'exhausted.' The proposition is that those males which would court for the longest time would show the 'high intensity' pattern of courtship, with long vibration bouts. In fact we obtained a linear regression of vibration bout length upon total response time which is significant at the 1% level.

The hypothesis therefore seems to be supported, and it is clear that, if it is true, we can regard the pattern of wild males as that typical of insects with high average motivation, that of yellows typical of low motivation. And this agrees with the impression obtained from the observations quoted above of low sexual vigor in yellow males.

The conclusion therefore seems to be that this gene mutation has in some way lowered the sexual motivation of yellow males. Just what this implies in physiological terms must be a subject for further investigation. Although our hypothesis was concerned with specifically sexual motivation we do not necessarily infer from this that the gene mutation has affected either some sexual hormone or some nervous threshold. It could of course have some peripheral effect upon an organ or tissue which indirectly affects sexual motivation. For instance it might diminish the amount of sperm in the gonads and thus reduce a stimulus (perhaps nervous) received from these organs. On the other hand it might affect the efficiency of the sense organs which receive stimuli from the female and thus reduce this source of motivation.

One could however imagine a much more general effect of the mutation. Perhaps it simply lowers physiological or mechanical efficiency, so that yellow males simply cannot keep up so well with rapidly moving females and thus are less stimulated by them. Hence again, their sexual motivation would be lowered. A further theory along these lines however could deny my hypothesis and suggest that sexual motivation is not involved at all. For it could be imagined that yellow

males simply can not vibrate their wings for such long periods as wild ones; perhaps they become fatigued after a few seconds and must then pause before they can vibrate again. Thus although impulses might be sent to the effector organs (i.e. the motivation might be high) the muscles might be unable to respond.

Certain points can be made about all these suggestions, although I have as yet made no experiments. The last idea can, I think, be denied at once by reference to the figures of table 3 where both males are courting *simulans* females. Both types of male give much longer vibration bouts to these females than to *melanogaster* ones. I have given reasons elsewhere (Bastock and Manning, 1955) why this should be so, but it is clear at once that yellow males are physically capable of giving vibration bouts of longer duration than those which they direct to *melanogaster* females. It could be suggested that yellow males are incapable only of the more difficult feat of vibrating for long periods while running and that perhaps *simulans* females run less often or less quickly, but I think this also unlikely. It may be true that *simulans* females run less often, but yellow males give long bouts of vibration to them even while running and it is at least not obvious that they run more slowly.

The idea that the gonads are affected is also made unlikely by the fact that yellow males seem able to fertilize successfully as many females in succession as do wild ones. Also Maynard Smith of University College, London, has found that mutant males of *D. subobscura* which lack a testis are just as successful in mating as wild ones (personal communication). This suggests that the gonads do not make a significant contribution to sexual motivation at least in this species.

The other suggestions remain as possibilities. The idea that yellow males are stimulated less because they fail to keep up so well with very active females is tempting in view of the fact that it is mainly while the female is running that the yellow males fail. However it is necessary to be cautious for it is clearly difficult to distinguish cause and effect in such a case. Careful investigation is necessary before it can be decided whether an insect is primarily inactive and hence poorly stimulated or whether it is primarily poorly motivated and hence less vigorous in pursuit. Many wild type males fail to keep up with females at the beginning of courtship yet quickly develop this capacity as they become 'warmed up.' In this case at least, the cause is most likely to be poor motivation.

The effect of this mutation upon the courtship behavior pattern may therefore be called 'direct' in the sense that it does not act by inducing the courted object to behave differently. However it is possible that it is 'indirect' in the sense that it may cause no change in the nervous system. The motivation may be altered because of some peripheral or even general physiological change. It would be interesting to know how many changes in behavior patterns could be and have been brought about by such indirect means, i.e. by changes in motivation attributable to a physiological or even morphological effect upon something other than the nervous system. Even the form of movements might be changed in this way. It has been shown by Tinbergen (1952) and others that many display activities have dual motivation. It is easy to imagine that were the strength of one of the sources of motivation to change, the form of the movement concerned might also change.

Evolutionary Implications

Finally the evolutionary implications of these findings merit some discussion. This gene mutation certainly brings about a change in the courtship behavior pattern of males which is very similar to differences found to exist between species and subspecies. However, it also has the effect of reducing the stimulating qualities of the males, at least as far as normal females are concerned. Clearly such a mutant, even although advantageous, would

have difficulty in establishing itself in a population. The most likely way in which a pure yellow population could arise, then, would be by the colonization of an isolated habitat, by, perhaps, a single yellow fertilized female. Alternatively, an already existing population, in a similar isolated niche, might be reduced by severe selection to a few yellow insects, supposing these to have some advantage in this situation.

The hypothesis that yellow (or any other mutant which has a similar change in its courtship) might, in some situations, be advantageous, is one which has never been seriously considered before. Yet positive evidence comes from Kalmus (1941), who shows that yellow insects are better able to withstand starvation in saturated air than are wild ones.

Thus it is by no means impossible that a pure yellow population could arise, differing in behavior from its parent population because the yellow gene (selected for other reasons) incidentally caused this. The fact that there are many observations of differences in mating vigor between species and subspecies of *Drosophila,* supports the idea that this sort of thing may well have happened. For example Mayr (1946) says that *D. persimilis* males are much more sluggish in their courtship than are those of *D. pseudoobscura.* Spieth (1952) gives many more examples in his review, and he points out that one cannot explain all these cases by assuming that laboratory conditions are more optimal for some species than others. For he points out that many of the examples show a reverse relationship between male courtship and female receptivity, and this suggests a 'gearing' effect rather than bad conditions.

This 'gearing' idea leads on to the proposition that an initial change in courtship behavior such as this might well stimulate the selection of other, perhaps more far-reaching changes in the behavior concerned. In my yellow *Drosophila* stocks changes in receptivity certainly seem to occur. When I first obtained these stocks it was clear that yellow females were much more highly receptive than wild ones. The percentage success in one hour which I obtained from pair matings was as follows:

wild male × wild female	62%
yellow male × wild female	34%
wild male × yellow female	87%
yellow male × yellow female	78%

Here the difference between the females is highly significant and on an analysis of variance ($P < 0.01$).

Later, however, I crossed my wild stock to my yellow stock for 7 generations, in such a way that the wild stock became genetically similar to the yellow except for yellow and closely linked genes.

The results of similar experiments were then as follows:

wild male × wild female	75%
yellow male × wild female	47%
wild male × yellow female	81%
yellow male × yellow female	59%

The female difference is no longer significant and it will be seen that by crossing to yellow stocks, I have raised the receptivity of wild females until it almost equals that of yellow females. High female receptivity is not therefore a characteristic of the yellow gene, and it is possible that it had been selected for in the yellow stock to counteract the effect of the deficient courtship.

However other changes might also or alternatively take place, for it might be dangerous in nature for females to become too highly receptive. Not only does this increase their chances of mating with males of other species but it prevents their operating the system of sexual selection (suggested by Richards (1927), Merrell (1949, 1953) and others) against males too inferior morphologically or physiologically to court adequately. If a male ceases to vibrate adequately it is possible that selection might favor females giving more weight to other stimuli (e.g. visual) received perhaps during orientation. In

turn this could favor the incorporation of more 'visual' elements into the courtship of the males. So the courtships of the 'new' population might progressively diverge from that of the old until the two might become sexually isolated.

Much of this is mere speculation, but there are indications to be found in comparisons of many closely related species which suggest that this may have occurred. For instance, *D. simulans* has a very similar courtship pattern to that of its sibling species *D. melanogaster*. The main difference is that *simulans* males are less active in their courtship than are *melanogaster*, and they tend to stand for long periods beside the female before they suddenly vibrate and copulate. However *simulans* females probably pay more attention to visual stimuli given in courtship, for mating success is considerably reduced in the dark in this species, unlike *melanogaster* (Spieth and Hsu, 1950). It is also interesting to find that *simulans* has added a new visual element to its courtship, which does not occur in *melanogaster*. To quote Spieth (1952) 'when the (*simulans*) male is in the act of circling the female, he often stops either directly in front of her or in front of one of her eyes and rapidly scissors both wings, gradually increasing the amplitude of the wings until they are extended 70° to 90° and then holds them briefly in this position, after which he drops the wings to the normal position and continues with his circling.'

All these differences could have evolved in the way which I have suggested above, stimulated by an original, perhaps incidental difference in courtship vigor. Admittedly, this is not the only possible explanation, but neither is this the only case of its kind. Spieth's review suggests many cases not only of differences in courtship vigor between species but also of differences in the sense modality given the greatest 'weight.' Moreover he finds that the most closely related species and subspecies tend to have very similar courtships but differ at the sensory level and he comes to the conclusion that sensory divergence nearly always precedes gross divergence in behavior pattern in *Drosophila*. This supports my hypothesis, if it can be shown that minor divergences in frequency or vigor usually accompany the sensory divergence. My contention is that the courtships described by him as 'similar' may well differ in a manner similar to that in which the courtship of the yellow *melanogaster* differs from that of the wild type.

The great interest behind this hypothesis lies in the fact that it suggests a way in which sexual isolation could evolve between two populations which remain in isolation from one another, and which, perhaps, have no differences which could cause their hybrids to be at a disadvantage and thus select for isolation on meeting. This possibility was first favored by Müller (1942, 1948) and later stressed by Mayr (1948). It contrasts with Dobzhansky's view (1951) that only vestiges of sexual isolation are likely to arise as automatic by-products of divergence, selection always being necessary to complete it. The evidence supporting Dobzhansky's view is that sexual isolation between *Drosophila* species is strongest where they overlap and becomes weaker as the distance between them increases (King (1947), Dobzhansky and Koller (1938)), but other workers, e.g. Wharton (1942), Mainland (1942), Spieth (1951), find apparently no such geographical correlation in other cases. Moreover the chief theoretical argument against the evolution of such mechanisms in isolation, is that they demand too many parallel and correlated changes, to have arisen without the stimulus of selection. If however one imagines that one small change could stimulate the selection of others, this difficulty is removed.

My conclusion, therefore, although speculative, is that this gene mutation in *Drosophila melanogaster does* change the pattern of courtship behavior in a manner which may well have been used in evolution. Such an effect of a single gene

mutation upon the frequency of elements in a behavior pattern has, I think, not previously been demonstrated. Although its effect is deleterious to mating success nevertheless it could be incidentally incorporated in an isolated population if the mutant concerned were useful in any other sense. It might then stimulate the selection of other divergences on behavior such as might ultimately bring about the sexual isolation of the population concerned, or it might alternatively form a basis upon which sexual isolation could be built by selection.

Summary

Yellow mutant males of *Drosophila melanogaster* have reduced success in fertilizing normal females.

There is a change in the pattern of courtship which could explain this. The wing display (vibration), which is shown to provide important stimuli perceived by the females' antennae, is given in shorter bouts and at longer intervals.

The idea that this behavioral change is indirect is dismissed. It is possible that females might react against changed color or scent, and, by repelling yellow males, thus influence their courtship. This is not supported by

(1) analyses of female behavior;
(2) comparisons of male behavior to *D. simulans* females which refuse both normal and yellow;
(3) comparisons of male success with females lacking visual or olfactory stimuli.

Consideration of the possible causal mechanism underlying the courtship pattern suggests that each of the three elements (orientation, vibration and licking) might occur at different thresholds of a fluctuating sexual excitation. A typical 'yellow' pattern with fewer and shorter bouts of 'higher' elements could be explained in terms of low average sexual excitation. This mutation thus changes a behavior pattern probably by affecting sexual motivation. How it does this is not yet known.

The nature of the change is of interest because it parallels behavioral differences found between closely related species of animals. Such a change may sometimes be incorporated incidentally into a population if the mutants concerned are selected for other reasons. A courtship change, such as this, might stimulate further evolution which could bring about the sexual isolation of the mutant population. For the reduction of an important stimulus from males might lead females to give weight to other stimuli (perhaps of another sense modality), which in turn would put a selective premium on other elements of the courtship.

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NOTES AND COMMENTS

FURTHER COMMENTS ON THE ORIGIN OF THE TETRAPODS

COLEMAN J. GOIN AND OLIVE B. GOIN

University of Florida

Our experience with migration and immigration of amphibians, while not too extensive, does seem to throw some light on the conditions under which amphibians abandon drying ponds and hence perhaps on the more basic problem of the first emergence of the vertebrates onto the land.

Romer (1945) has suggested that the tetrapod limb is an adaptive modification permitting primitive aquatic amphibians confined in some drying pool of the Devonian landscape to leave this pool in search of asylum in a larger body of water. Orton (1954) argues that this is contrary to experience since, if the lands are drying, the amphibians tend to congregate in the dampest spots available rather than to disperse across the arid and inhospitable terrain. She suggests that the tetrapod limb originated as a burrowing device to permit estivation during times of drought and that the amphibians emerged on land in the Mississippian when more humid conditions prevailed. Ewer (1955) points out that this thesis fails to answer the question of why the amphibians should have left the water at a time when aquatic habitats were expanding. He discusses three different patterns of behavior by which the South African Clawed Toad, *Xenopus laevis,* has responded to the drying of its normal habitat. Usually the toads estivate by simply burrowing in the mud. As Ewer points out, this is in keeping with Orton's suggestion that the limb was developed as a fossorial device. As a second behavior pattern he cites some *Xenopus* emerging from mud that had been cleared out of a duck pond and spread over the neighboring lawns. When the rains came, the toads emerged from the softening mud but simply wandered aimlessly about until most of them had died from exposure to the sun. This, of course, was an artificial situation. The third type of behavior was shown when thousands of toads left an impoundment that was commencing to dry and migrated across land to a nearby river.

Regarding this last behavior pattern, Ewer suggests the probability that such mass migrations occur in response to population pressure induced by the shrinking of aquatic habitats but that they take place only under favorable climatic conditions. He offers no hypothesis as to what the requisite climatic conditions are.

Gunter (1956) suggests that the tetrapod limb evolved gradually in the water as the crossopterygian fin was used first as a prop and then as a means of 'walking' in shallows and across mud flats to enable its possessor to escape enemies or feed on terrestrial arthropods. He believes that the invasion of the land came as Romer and Ewer suggest in response to the drying of aquatic habitats but only after the tetrapod limb had already developed.

In the woods behind our house near Gainesville, Florida, are two ponds which from time to time go dry during periods of drought. In our back yard we have an artificial pool which we keep filled with water. A number of amphibians live around and breed in the ponds back in the woods but by far the two most numerous ones are the Southern Cricket Frog, *Acris gryllus dorsalis,* and the Bronze Frog, *Rana clamitans clamitans.* When a dry spell ensues, both species concentrate in the vicinity of the residual pools. This concentration brings about an increasing population pressure as the habital area is reduced in size. Release from this population pressure depends, not on the surrounding regions becoming sufficiently moist to attract invasion, but *only on their becoming sufficiently moist to permit dispersion.* There is quite a difference between the two. Thus *Acris* and *Rana* do not await the termination of a drought by sufficient rain to form new ponds to attract them before they start moving out. Enough rain to produce temporarily humid conditions on the ground and in the air permits them to respond to the migratory urge for after such a rain both species show up at our yard pool. Although we do not know the source of the population, the same thing seems to apply to the arboreal frog, *Hyla squirella.* The young of this species invade our yard each fall after the breeding season. We have found that invariably the larger waves of migration occur on nights when there has been a light rain, which apparently permits the young of this rather delicate species to cross terrain that at other times would be much too dry for them.

It may be urged that during a time of drought most of the other aquatic environments in a region would also have been subject to shrinkage so that the first amphibians to leave their ponds would have had little chance of finding more favorable habitats. One of the attendant evils of overcrowding is the increased competition for the available food supply. If the first amphibians to move out on the land were able

to supplement their diet by feeding on terrestrial arthropods, their chances of survival would have been materially increased. Romer believes that feeding on land was not of much importance to the first amphibians since they were fish-eating forms. However, many primarily piscivorous fishes will also bite at other objects—worms, insects and other small invertebrates. Furthermore, the young of such fishes subsist for some time on small aquatic arthropods and only adopt a piscivorous diet after they have attained a certain size. It may be significant that the urge toward movement away from the ponds seems to be most strongly developed in young frogs. Almost without exception the ranas that have reached our pool have been immature. Moreover, our yard has from time to time been invaded by large numbers of the young of such other frogs as *Scaphiopus holbrooki, Microhyla carolinensis* and *Hyla crucifer*.

Ewer's emendation of Romer's hypothesis is thus that the primary stimulus toward migration overland in the first amphibians was increased population pressure induced by the shrinking of aquatic habitats during drought. We suggest that this stimulus was released by rainfall sufficient to moisten the surrounding terrain but not sufficient to fill up the ponds and thereby relieve the overcrowded conditions. We further suggest that the ability to utilize the food resources of the land may have had survival value once the all-important first step away from the water had been taken. Given the tendency to disperse overland under such conditions and the ability to sustain life on land the chances of the first amphibians finding more adequate aquatic habitats would have been materially improved.

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Manuscripts should be addressed to

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